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TABLE OF CONTENTS

Lewis Ralph Jones, 1864–1945 ..... G. W. Keitt and F. V. Rand 1
Internal cork, a new disease of sweet potato of unidentified cause. . C. J. Nusbaum 18
Effect of storage conditions on survival of Colletotrichum gossypii. C. H. Arndt 24
The internal infection of cotton seed and the loss of viability in storage. . C. H. Arndt 30
Some factors influencing curly top virus concentration in sugar beets. N. J. Giddings 38
Mass action as a factor in curly-top-virus infection of sugar beet. N. J. Giddings 53
Stripe smut (Ustilago striaeformis), in relation to bluegrass improvement ..... J. G. Leach, C. V. Lowther, and Mary A. Ryan 57
Tatter leaf of sweet cherry ..... R. S. Willison and G. H. Berkeley 73
Announcement 85

PHYTOPATHOLOGY

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Official Organ of The American Phytopathological Society

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LEWIS RALPH JONES
1864–1945

LEWIS RALPH JONES
1864–1945

G. W. KEITT AND F. V. RAND[1]

Lewis Ralph Jones, Professor Emeritus of Plant Pathology of the University of Wisconsin, died at Orlando, Florida, on April 1, 1945, in his eighty-first year. In his passing the University of Wisconsin has lost one of her greatest scholars, the American Phytopathological Society one of its founders and leading spirits, and biological science one of its most constructive leaders. His many students throughout the world have lost a beloved teacher and trusted friend.

His forebears were vigorous and able pioneers in the settlement of this country. His father, David Jones, born of English parents in 1828 at the border of England and Wales, came to America with his family at the age of fourteen. After three years at Utica, New York, the family settled on a farm near Kenosha, Wisconsin. At the age of twenty, David bought a tract of government land in Metomen township of Fond du Lac County, near the site on which the village of Brandon later was established. Here he built his house and lived a long and useful life as a successful farmer and a leader in the community. He raised Guernsey cattle, and his oats won a bronze medal at the St. Louis Exposition. In 1856, he married Lucy Jane Knapp, who was descended from a long line of New England and Pennsylvania Dutch ancestors, some of whom became pioneers in western Vermont soon after the Revolutionary War. Lucy Jane, daughter of Alva and Mary Cuthbert Knapp, was born at Starksboro, Vermont, May 27, 1837. In 1850, her parents moved to Metomen township, and later to Brandon. Before her marriage she was teacher of the local district school. She was a woman of much wit and charm and a discriminating reader with broad interests. From girlhood in one of the most picturesque of the Green Mountain valleys she brought a love of nature, and especially of plant life, that she early imparted to her children. She was devoted to her family and to the cultural and religious life of the community. Hers was the dominant influence in making it possible for her son, Ralph, who early showed the bent of a scholar, to prepare himself for a liberal education.

Lewis Ralph, the third of six children of David and Lucy Knapp Jones, was born on December 5, 1864. In their busy home of high ideals and sterling values were laid the foundations of the character that was to guide him so well. His early education was at Brandon. From 1883 to 1886 he attended Ripon College, where he became attracted to biological science under the stimulating influence and helpful counsel of C. Dwight Marsh, Professor of Chemistry and Biology. For more advanced studies he went

[1] Grateful acknowledgments are made to colleagues in the Department of Plant Pathology of the University of Wisconsin and to many other former students and friends of Professor Jones for their assistance in the preparation of this sketch of his life and works.

in 1886 to the University of Michigan. His undergraduate education was oriented towards medicine, but in his senior year he decided to go into plant pathology instead. In making this decision, he was much influenced by Dr. Volney M. Spalding, Professor of Botany, and Erwin F. Smith, then a mature and exceptionally brilliant graduate student who was already well started in his distinguished career in the United States Department of Agriculture. In his last semester as an undergraduate he was privileged to attend Smith's doctoral examination. In his own words: "This examination covered Smith's early work on peach yellows. The glimpses thus given me of the significance of and opportunity for research in the field of plant pathology were most inspiring. . . . In conference with my wise and revered counselor, Professor Volney M. Spalding, the decision to turn to plant pathology was promptly reached and never since regretted."

The arresting fact that the University of Michigan should at this time have launched upon their careers two young men who were to become outstanding leaders of their generation in both plant pathology and the broader field of biology and agriculture suggests some consideration of background events. Great developments in American education and research were in the making, the University of Michigan was becoming one of the foremost centers of learning in this country, and the pioneer homes of the Middle West were producing young men with the character, ideals, intellect, vigorous initiative, and sympathetic understanding of needs that enabled them to meet the great calls for service that so rapidly developed.

Plant pathology in America was in its infancy at a time when a rapidly expanding agriculture was presenting plant disease problems of great urgency. The foundations of this new science had been laid in Europe by such pioneers as Prévost, the Tulasnes, de Bary, and Kühn, and similar advances had been made in the field of infectious diseases of animals and man by Koch, Pasteur, Lister, and others. Farlow had studied under de Bary in the mid-seventies and brought the inspiration and methods of this great leader to Harvard, where he had begun training men, especially in the taxonomy and life-histories of parasitic fungi. Burrill, at the University of Illinois in 1878 to 1881, had made the classic discovery that bacteria incite the fire blight disease of apples and pears, and pioneer work on plant diseases was under way in many states. By 1885, Millardet and others in France had discovered Bordeaux mixture and demonstrated such dramatic success in its control of destructive plant diseases that great impetus had been given to the development of plant pathology and related fields of agricultural science. A section of mycology, devoted primarily to work on plant diseases, had been established in the U. S. Department of Agriculture in 1886, and in 1887 the Hatch Act had made possible an agricultural experiment station in each state.

In the mid-eighties, the University of Michigan was rapidly developing in scientific strength and leadership. Dr. Victor C. Vaughan was beginning the program in medical bacteriology that he and Dr. Frederick V. Novy

continued with such success. Professor Volney M. Spalding sensed the great needs and opportunities in the new field of plant pathology and encouraged his students to work on plant diseases. Once they had the opportunity to engage in such studies and envision the needs for service and the opportunities for scientific contribution in this field, it was inevitable that men with the splendid natural aptitudes and fine spirit of Smith and Jones should enter it with all their energies and enthusiasm.

Jones' first teaching experience came between his junior and senior years, when he served as Instructor in Natural Science at Mt. Morris Academy, Mt. Morris, Illinois, for the year of 1887 and two terms of 1888. The success of this early teaching is attested by the life-long friendship of members of his classes.

Upon completing his course and receiving the Ph.B. degree in 1889, he was called to the University of Vermont, where he served as Botanist of the Agricultural Experiment Station, 1889–1910, Instructor in Natural History, 1889–91, Assistant Professor of Natural History, 1891–92, Associate Professor of Natural History, 1892–93, and Professor of Botany, 1893–1910.

On June 24, 1890, he married May I. Bennett, a classmate at Ripon College. She shared generously in his life's effort, making many personal sacrifices to help him meet the urgent pressure of work during his early and middle years and keeping their hospitable home always open to his students and other friends. She passed away peacefully in her sleep on September 22, 1926.

His twenty years at the University of Vermont, interspersed with travel and special studies at the University of Michigan, in the United States Department of Agriculture, and in Europe, marked the first stage of Professor Jones' career and established him as a leader in American botany and plant pathology. In this early formative period in the development of the state agricultural experiment stations, the United States Department of Agriculture, and the state colleges and universities, his contribution was rich and varied. Most men are fortunate if they can render distinguished service as teacher, investigator, or administrator and builder in education and research. Professor Jones made distinguished contributions in all of these fields.

He became one of the most effective and beloved teachers in the history of the University of Vermont. He was held in the highest affection by his students and so great was his esteem by the student body that many who were concentrating in unrelated fields took work with him to experience the influence of his tutelage and personality. Some of these were so attracted that they continued in his field for their life work. During his last few years at Vermont over a score of students went out from his laboratories into professional work in plant science.

He was highly productive in research and in its practical applications, developing a national leadership in studies of the nature and control of plant diseases. His first important contribution was his pioneering in the

adaptation of Bordeaux mixture to use in America. Beginning with experiments in the summer of 1889, in which late blight of potato was successfully prevented, he rapidly developed methods for the practical control of that disease and of both apple and pear scab by this "marvelous new French fungicide." Examination of the appended bibliography will give some idea of the wide scope of the work that followed. The practical needs of agriculture and forestry were given all possible consideration. Duties that in other hands might have been routine were made to yield high educational values, and contacts with the farmers were made to contribute both to the solution of their problems and enlistment of their interest and cooperation. Notwithstanding the multiplicity of duties, certain problems were selected for intensive researches that resulted in contributions of the highest quality. Notable among these were his investigations of potato diseases, bacterial soft rot of vegetables, and sap flow in sugar maple.

The work on potato diseases included studies at the University of Michigan and in Europe, where from April to September of 1904 he visited leading laboratories in England, France, Belgium, Holland, and Germany and became acquainted with many of the foremost plant scientists and plant culturists of those countries. Commissioned by the United States Department of Agriculture to make collections of potato varieties that breeders of this country might use in the endeavor to develop stocks resistant to disease and superior in other qualities, he brought back over 90 varieties. These were studied at Vermont in association with Professor William Stuart, and turned over to Professor Stuart for further studies when the latter became leader of the potato breeding program of the United States Department of Agriculture. The classical paper on late blight of potato published in 1912 by Jones, Giddings, and Lutman as Bulletin 245 of the Bureau of Plant Industry remains the most comprehensive and authoritative publication on this important disease.

Another notable experience during the Vermont years was the six months spent in 1899 in Erwin F. Smith's laboratory in Washington, when at Smith's invitation he took his problem on bacterial soft rot of vegetables there for special studies. This was a very fortunate and significant experience for all concerned. It enabled him to renew his associations with Smith and brought him into close contacts with a group of outstanding leaders in the development of the Bureau of Plant Industry. Community of interest drew them together in informal seminars that met frequently in one or another laboratory. These were supplemented by lunching together and by "Dutch" dinners at Harvey's famous seafood restaurant. Besides Smith and Jones, regular attendants included A. F. Woods, B. T. Galloway, M. B. Waite, Mark A. Carleton, and Theodore Holm. These informal seminars later developed into the Botanical Seminar of the Bureau of Plant Industry and finally into the Botanical Society of Washington. In recalling these early experiences, Dr. Woods recently said: "The discussions were chiefly along bacteriological and physiological lines, and Jones was one of

the leading spirits. He was looked upon by the Department as a prize visitor. He brought to the group the university point of view. Though he came for further bacteriological training and experience as well as research, he gave fully as much as he got. We were proud to have him with us." Out of such relations grew mutual understanding and confidence, and the way was paved for life-long associations of great advantage to all concerned.

A further outcome of the investigations on bacterial soft rot of vegetables was the publication of Prof. Jones' classical papers describing *Bacillus carotovorus* and showing the mechanism of its incitation of rotting by production of the enzyme, pectinase, which dissolves the middle lamellae of the cell walls of the host plant.

In 1901 and 1904 periods of graduate study were spent at the University of Michigan, from which he received the Ph.D. degree in 1904.

Professor Jones' influence at Vermont extended far beyond the boundaries of his special field. He was a trusted counselor, both of students and colleagues, and a leading spirit in the life of the University. He was the leading organizer of the Vermont Botanical Club and the initiator and a President of the Vermont Forestry Association. The L. R. Jones State Forest of Vermont was named in recognition of his services to forestry. He was a leader in the movement that established training in home economics at Vermont, and was highly instrumental in starting teacher training work there. It is a tribute to his accomplishments as a teacher that he was asked to serve as the first Dean of the Department of Teaching. This assignment was carried in addition to his other work.

In 1909 Professor Jones was called to the University of Wisconsin to found its Department of Plant Pathology. Difficult as it was to leave the friends and associations in Vermont, the decision was made easy, to use his own words, . . . "since we could by returning to Wisconsin personally renew our early home associations and professionally meet the increasingly evident responsibility of opening thus, in a larger university particularly strong in botanical traditions and associations, opportunities for the training . . . of younger men and women beyond those which had been possible to us of the older generation." To his new task, which he took up on February 1, 1910, he brought the rare talents and devotion that were to win him recognition, not only as the dean of the plant pathologists of his generation, but also as one of the leaders of his time in the broad field of biology and agriculture.

He combined the highest ideals of scholarship with a first-hand understanding of the practical needs of agriculture and a vital interest in the applications of science to the service of mankind. He was rarely gifted in understanding and inspiring both his students and his colleagues, and gave of himself without reservation. He had the vision and the ability to integrate his new department closely with related departments of the University and with the U. S. Department of Agriculture, always fostering mutual

assistance and cooperation. From the beginning it was his policy, insofar as possible, to send his students for basic training to the related departments concerned, developing in plant pathology only research and such special courses as were deemed essential to supplement the basic training. Because of the close natural relationships of the two fields and the strength and cordial cooperation of the Department of Botany, work in plant pathology and botany was especially closely integrated. Much of the strength of the development in plant pathology was made possible by the splendid cooperation of Dr. R. A. Harper and his colleagues in the Department of Botany from the outset and by their successors throughout the years.

Professor Jones conceived of research as the central activity about which to build his department. Only by competent research on the basic problems of plant disease development and control could the practical needs of agriculture be met. Only upon the results of such research could adequate instruction be based. He knew that students learn best by doing, and early recognized the great advantages of closely integrating postgraduate teaching with the research program of the department. Never desirous of ambitious buildings or unnecessarily large appropriations, whose dangers he well recognized, he was ever earnest in urging provision of the facilities and funds necessary for the effective and economical development of the work of the department, and was exceptionally gifted in bringing together support from different sources for attack on common problems.

Students came to his laboratory from all over the world and have gone forth to most of our states and to many foreign lands with the benefits of his training and the stamp of his character. Though his objective was always quality rather than quantity, his department has trained 145 students as majors in plant pathology who have been awarded the Ph.D. degree by the University of Wisconsin. Professor Jones believed strongly in the value of the ancient custom of migration of students, and encouraged it, both in welcoming those from other institutions and countries and in advising his own students to broaden their horizons by studies elsewhere. Similarly, no opportunity was missed to encourage exchange of visits with qualified colleagues from other institutions.

Though the time that Professor Jones could devote to his individual researches in the years at Wisconsin was progressively limited by the multiplicity and weight of his other responsibilities, the quality of his work set a standard for all. In developing the research program, as in other lines of building the department, it was his method to select men carefully, assign their responsibilities, and then give them all possible freedom, support, and encouragement in their work. He realized that what he could accomplish by aiding in the development of men, programs, and institutions would greatly outweigh any personal research contribution he might hope to make. Furthermore, his greatest satisfaction was in assisting his students or colleagues to find themselves and in seeing their growth and accomplishments. He was scrupulously careful to see that all possible deserved credit went

to his students and associates. His contribution as director of the research work of his department and as a leader and counselor in the development of trends of research was of the highest importance. Notable examples, both of his personal research and his leadership in trends of research during the Wisconsin period, are his contributions to the study of disease resistance in plants, including development of disease-resistant varieties, and his work on the influence of environment on plant diseases. The late Dr. E. J. Butler,[2] distinguished Director of the Imperial Mycological Institute (Kew, Surrey, England), pointed to the high-light of Professor Jones' contribution as follows: "There are three phases in the history of plant pathology: First, the period of de Bary, in which the fungus held first place; second, the period in which the host received most attention; and finally, the present period in which disease is considered as an interaction of both under the conditioning influence of the environment. The leader in this is Jones."

The development of his department was but one of Professor Jones' great contributions during the Wisconsin phase of his career. His knowledge and experience, breadth of vision, wisdom, and rare constructive abilities were constantly in demand.

He contributed generously to the broader aspects of the development of the University. He was one of the founders and leading spirits of the Graduate Biological Division, a faculty group spontaneously organized to foster graduate work and related interests in the field of the biological sciences. He served on major committees of the University dealing with institutional policies, and his counsel was widely sought and highly regarded.

His contributions outside the University were varied and highly significant. One of special interest to readers of Phytopathology was his services to our Society, its members, and its journal. He was one of the founders and charter members of the American Phytopathological Society, its first president (1909), the first editor-in-chief of Phytopathology (1911–14), and a life-long inspiring leader in the work of the Society. He contributed largely to the rapid and sound development of the Society and to the vitality, practicality, and generous spirit of cooperation and service that have characterized it.

Professor Jones was also one of the leading spirits in the Botanical Society of America. He served as vice-president in 1910 and president in 1913. He was a member of the committee appointed to establish a journal for this society (1913) and served on the Editorial Committee of the American Journal of Botany (1914–16 and 1919–21).

At the end of the first world war, when many major responsibilities for scientific leadership shifted from Europe to America, Professor Jones' school of plant pathology at Wisconsin was fully established and he was ready and able to contribute largely to leadership in the broad field of biology and agriculture. He rendered valuable services through his rela-

[2] From remarks made at the banquet given in appreciation of Professor Jones by his former students at Ithaca, N. Y., August 19, 1926, as recorded by F. V. Rand.

tions in the National Academy of Sciences and the National Research Council, serving in the latter as vice-chairman of the Division of Biology and Agriculture from 1919 to 1921 and chairman in 1922. He was one of the founders of the Tropical Plant Research Foundation and served as president from 1924 to 1943. He was one of the organizers of the Boyce Thompson Institute for Plant Research and served on its Board for the rest of his life. In 1943 he was appointed by President Roosevelt to the Science Advisory Board.

There were many other special services or missions during these busy years. He found time while president of the Tropical Plant Research Foundation to go to Cuba as adviser on problems of sugar-cane culture. In 1926 he went to the Hawaiian Islands as consultant on problems of pineapple culture. He visited Puerto Rico in 1930 as adviser on agricultural developments. In 1930, he made an extensive tour of Europe and European Russia, visiting leading laboratories, conferring with colleagues, and renewing associations with his former students. This visit included participating in the Fifth International Botanical Congress (Cambridge, England), where he served as Chairman of the Section of Mycology and Plant Pathology. In 1931 and 1932, he traveled extensively with similar purposes in the Far East and the Islands of the Pacific, with special attention to Japan, Korea, China, the Philippine Islands, and Hawaii.

Professor Jones had the richly deserved good fortune to be greatly appreciated during his lifetime, receiving many honors at home and abroad. A list of his affiliations and honors is given on a later page.

An unusual honor that touched him very deeply was the reunion banquet given in his appreciation on the occasion of the presentation of his portrait to the University of Wisconsin by his former students, at the International Congress of Plant Sciences at Ithaca, N. Y., on August 19, 1926. High tributes for his many-sided contribution were paid by W. A. Orton, A. G. Johnson, I. E. Melhus, H. A. Edson, and G. W. Keitt, representing his former students, L. H. Bailey, R. A. Harper, and Erwin F. Smith, representing colleagues in the United States, and O. Appel and E. J. Butler, representing colleagues abroad. With the portrait were presented 22 volumes of the collected works of his students since 1910 to supplement the seven volumes that had been presented him when he left the University of Vermont. It is unfortunate that limitations of space prevent inclusion of an account of the substance of these tributes and of Professor Jones' response. It must suffice to include his closing remarks, which so well illustrate his ideal of service through science.

"Watching the trend in modern scientific developments as I have, I wish to warn you of one of the subtlest dangers facing you collectively as well as individually. I refer to the danger of professionalism. If you understand my meaning I think you will agree that with the present outlook, and especially in a young, rapidly developing country like ours, it is impossible to escape this danger altogether. You Vermont boys need only

to revision that great amateur botanist, Dr. Pringle, to understand what I mean by the spirit of amateurism as compared with professionalism—the devotion of one who is working for the love of science rather than for a living through science. And in Wisconsin we are happy in having another such in Dr. Davis. Unfortunately, the temptation to time-serving professionalism is stronger today than it was a generation ago, when, for example, in the entire membership of the Vermont Botanical Club I was perhaps the only one who was paid for studying plants.

"But, fortunately, on the other hand, the objectionable aspect of professionalism need not follow simply because one receives a living wage as a botanist. Quite as fortunately, the dangers are in no wise proportioned to the size of the salary. It all depends upon one's attitude toward his work. I have no fear as to this if we can only continue in the spirit in which I am sure you all have begun—the spirit of devoted scientific service. I like to think of those in state or federal positions in plant pathology as holders of honorary fellowships, as it were, for public service to society through creative work in the field of the plant sciences. In this way the best of that characteristic of the amateur spirit may be adequately perpetuated in our professional group, let it grow to any size and sub-divide to any degree of specialization."

The pressures of Professor Jones' scientific responsibilities were not allowed to mar the graciousness of his life. He was the kindest and most considerate of men, ever available to his students and his colleagues for wise and sympathetic counsel. His students were always welcome in his home, and many of their happiest memories center about his hospitable fireside. Always keenly interested and active in the cultural, civic, and religious life of his community, he was a member of the University Heights Poetry Club, the Madison Literary Club, the University Club, the Rotary Club, the Masonic Fraternity, and the Congregational Church.

On July 27, 1929, he married Anna M. Clark, one of his former students at Vermont, who survives him. A distinguished teacher in her own right, she shared fully in his interests, friendships, and hospitality; and after the condition of his health required restriction of activities her loving care and congenial companionship made his twilight years very happy ones.

In 1930, Professor Jones resigned the chairmanship of his department and asked to be put on part-time service in the University, in order to free additional time for travel and his outside scientific responsibilites. He retired from active service in 1935, but continued to carry important responsibilities in the National Academy of Sciences and as committee man, trustee, and counselor. The leisure of his later years permitted much of the travel that he and Mrs. Jones so much enjoyed. Always central in their travel plans was the continuation of his long-established custom of visiting colleagues and former students, ever radiating encouragement, inspiration, and good cheer. Except when interrupted by more extended travels, the winters were usually spent in Florida and the summers at Mrs. Jones'

ancestral home in Brookfield, Vermont, with a spring visit to Madison. In the winter of 1941, Professor Jones developed a heart condition that necessitated some restrictions of his activities. Nevertheless, he continued to enjoy very good general health and retained his keen interests and kind and happy associations to the last.

An event that touched him very deeply and is a lasting satisfaction to his many friends was the receipt upon his eightieth birthday of some 250 letters of affection and appreciation from former students and other friends from all over the world.

He passed away peacefully in his sleep during the night of March 31, 1945. He had been much interested for several days in formulating plans with Mrs. Jones for the coming months. Apparently he had no premonitions and suffered no pain.

Funeral services were held at the Congregational Church at Madison, of which he had been a member for 35 years, and interment was in the family lot at Brandon.

Though his personal researches were of the first rank, Professor Jones' greatest contribution was the impress he made on the lives of others and the constructive influence he exerted on institutions and programs of education and research in a critical formative phase of their development in North America. What he did was possible only because of what he was. His integrity and unswerving devotion to the highest ideals of science and of life, his clarity of vision and steadfastness of purpose, his deep and sympathetic understanding of human relations, and his unfailing kindness and untiring devotion to the service of others were a lasting inspiration to those who had associations with him. He will long be remembered for his leadership in science and his example in life.

DEGREES, HONORS, AND MEMBERSHIPS

University of Michigan, Ph.B. (1889), Ph.D. (1904), LL.D. (1935); University of Vermont, Sc.D. (1910); University of Cambridge, Sc.D. (1930); University of Wisconsin, Sc.D. (1936).

Editor-in-chief, Phytopathology (1911–14); editor, American Journal of Botany (1914–16, 1919–21), bacteriological terms in Webster's New International Dicitionary of the English Language (1928); trustee, Boyce Thompson Institute for Plant Research (1924–45); president, Tropical Plant Research Foundation (1924–43); an honorary president, Third International Congress for Microbiology (New York, 1939).

Member, Academy of Natural Science of Philadelphia, American Association for the Advancement of Science (fellow; vice-president and chairman of Section O, 1924–25), American Philosophical Society, American Phytopathological Society (president, 1909), American Society of Naturalists, Association of Applied Biologists (England), Board of Park Commissioners, Burlington, Vt., Botanical Society of America (vice-president, 1910; president, 1913), Fifth International Botanical Congress (chairman,

Section of Mycology and Plant Pathology), Vermont Botanical Club, Vermont Forestry Association (president, 1908), National Academy of Sciences, National Research Council (vice-chairman, Division of Biology and Agriculture, 1919–21; chairman, 1922), New England Botanical Club, Phytopathological Society of Japan (honorary), Science Advisory Board (appointed by the President of the United States in 1934), Société de Pathologie végétale et Entomologie agricole de France (honorary), Verein für Angewandte Botanik (honorary), Wisconsin Academy of Sciences, Arts, and Letters, Gamma Alpha, Phi Kappa Phi, Phi Sigma, Sigma Xi (president Wisconsin chapter, 1914–15), Madison Literary Club, University Heights Poetry Club (Madison, Wis.), University Club (Madison, Wis.), Cosmos Club (Washington, D. C.), Rotary Club, Masonic Fraternity, Congregational Church, Republican Party.

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INTERNAL CORK, A NEW DISEASE OF SWEET POTATO OF UNIDENTIFIED CAUSE[1]

C. J. NUSBAUM

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INTRODUCTION

In the spring of 1944 the occurrence of small dark spots was observed in the flesh of a few sweet potatoes (*Ipomea batatas* (L.) Lam., Porto Rico variety) at the Edisto Experiment Station, near Blackville, S. C., as they were being cut and examined for flesh color. At first the trouble seemed inconsequential and little attention was given to it. A few days later, however, the same trouble was noted in a stored lot of sweet potatoes near Orangeburg, S. C. Following this observation, approximately 300 stored roots were selected from Edisto Station stocks and each root was cut in thin sections and carefully examined. A trace of spotting was found in about 20 per cent of the roots. Further observations during the 1944 harvest season supported the view that a new and potentially serious disease of the sweet potato existed. The purpose of this report is to describe the symptoms of the disease, herein referred to as "internal cork," to give a preliminary account of its occurrence in South Carolina and to discuss certain unsuccessful attempts to determine its etiology.

SYMPTOMS OF INTERNAL CORK

The symptoms of internal cork are distinctive and are not likely to be confused with other types of sweet-potato flesh degeneration. The corky spots may occur singly or in groups at any point in the fleshy tissues. There are no external signs except that cavities may form at points where spots occur near the surface, as shown in figure 1, A. Numerous cases were observed, however, where spots at the periphery of the root failed to cause cavity formation (Fig. 1, B). Although many spots have been observed in the cortical zone just beneath the skin, none have ever been found erupting to form a surface lesion. Therefore, in most cases the disease can only be detected by cutting the roots and, even then, it may be overlooked unless the roots are cut in thin slices. The affected tissues, as shown in the longitudinal sections in figure 1, A, B, and D and in cross sections in C, are irregular but definite in outline. In cross section the spots may reach a diameter of about 3 cm. and rarely exceed 5 cm. in longitudinal section. Where several are grouped together, however, a considerable portion of the root may be involved. Such a case, the most severe yet noted, is shown in figure 1, D. The affected tissue is dark brown or sometimes nearly black and is hard and corky. After exposure to the air for several hours the color generally fades to medium brown. After several days' exposure, desiccation and subsequent

[1] Technical contribution number 127 of the South Carolina Agricultural Experiment Station.

shrinkage of surrounding healthy tissues gives the spots a raised or bulged appearance. Internal cork tissues remain firm during cooking. If they are crushed between the teeth, a very slight bitter taste can sometimes be detected but the flavor of the surrounding healthy tissue is not noticeably affected.

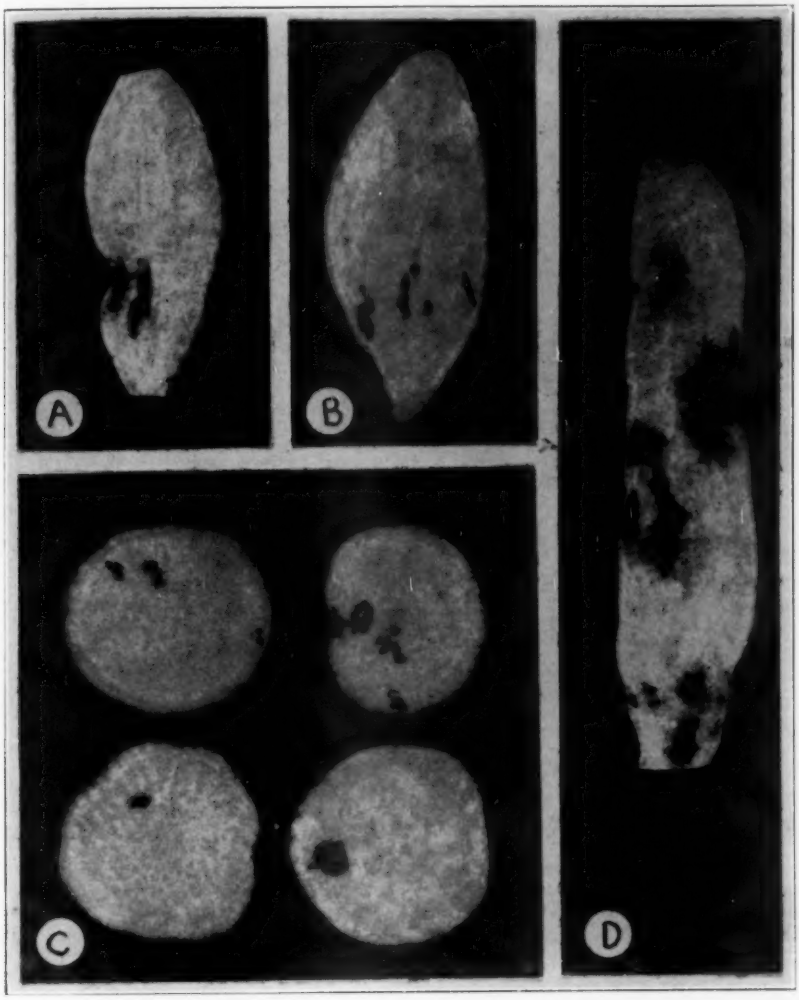

FIG. 1. Internal cork of sweet potato in roots of the Porto Rico variety. A. Longitudinal section showing a surface cavity associated with a corky area located at the periphery. B. Longitudinal section showing a peripheral spot without a cavity. C. Cross sections showing the typical random distribution of internal cork in the fleshy tissues. D. A longitudinal section of a severely affected root. Note the extensive necrotic areas which are probably aggregates of many individual spots.

Microscopic examination of sections of corky spots gave some indication of their nature. Bits of sweet-potato flesh containing corky spots were fixed in a formol-acetic-alcohol mixture, hand-sectioned, stained with Delafield's haematoxylin, and mounted in Euparal. The smallest spot examined was

composed of a necrotic area, 5 or 6 cells in diameter, located in parenchyma tissue. Larger spots (Fig. 1) showed an irregular central area of collapsed cells surrounded by a phellem layer several cells thick. Cell walls in this layer stained red with Sudan IV, which indicates presence of suberin. The remains of disorganized xylem vessels sometimes were found in the necrotic area. The time of appearance of necrosis, the exact point of its initiation or the probability of its development in storage has not been ascertained.

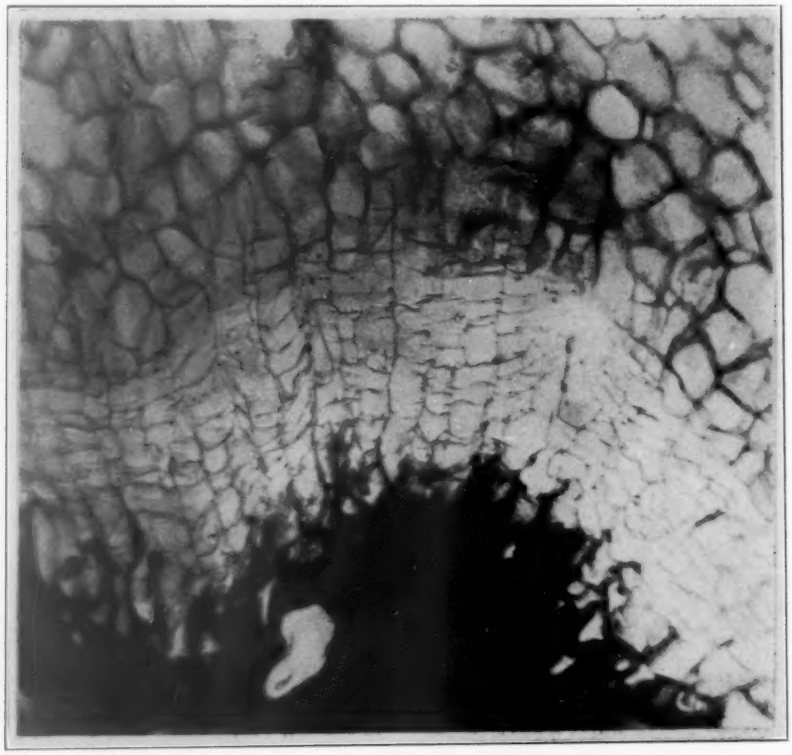

FIG. 2. A photomicrograph of a cross section of flesh tissue of a Porto Rico sweet-potato root, showing the marginal area of an internal cork spot. The central necrotic area, at the bottom, is separated from the healthy parenchyma tissue, at the top, by a phellem layer.

OCCURRENCE

During the 1944 harvest season samples of sweet potatoes were examined on many farms throughout the coastal plains region of South Carolina. Internal cork was found in nearly every locality visited, although it usually occurred only in trace amounts. The disease was observed in the roots of 38 different varieties and strains of sweet potatoes on trial at the Edisto Experiment Station. It was also found indiscriminately in sweet potatoes harvested from numerous experimental plots where various cultural and nutritional treatments were under investigation. A random sample of 480 roots of the Porto Rico sweet potato was collected from several fields and each root was examined and classified as to the incidence of internal cork. Thirty-nine

per cent of the roots were free from cork, 28 per cent had a trace, 25 per cent had 5 or less spots per root, and the remaining 8 per cent contained more than 5. It was judged that about half of those in the last category, or about 4 per cent, would provoke an adverse consumer reaction. The disease was observed on the trimming belts at 3 dehydration plants, although it was present in such small amounts that it apparently escaped the attention of the operators.

ETIOLOGICAL STUDIES

The possible existenee of a pathogenic organism in internal cork tissues was first investigated. Forty-four stored Porto Rico roots, which showed internal cork on sectioning, were surface disinfected. Then bits of corky tissue and adjacent flesh were removed aseptically and plated on potato-dextrose agar. Forty-one of the plates remained sterile throughout a three-weeks period. The remaining 3 plates showed colonies of an unidentified *Fusarium*. According to Dr. G. M. Armstrong,[2] however, it is not uncommon that Fusaria are recovered from apparently healthy sweet-potato tissue. Similar culture of corky tissue from 35 freshly harvested roots on both potato-dextrose agar and sweet-potato-decoction agar in the fall of 1944 gave negative results in every case. Consequently the possibility that internal cork may be caused by a culturable pathogen was dismissed.

The possibility that internal cork might be due to boron deficiency was suggested by the resemblance in many respects of internal cork symptoms to those of internal black spot[3] of garden beet, a boron deficiency disease. Therefore, sweet potatoes, grown in field plots which received applications of borax at rates of 0, 5, 10, 20 and 30 pounds per acre, respectively, in combination with 3 levels of potassium on both limed and unlimed soils, were examined. Although the plants had responded to the borax treatments and had symptoms ranging from severe deficiency to extreme toxicity, there was no correlation between reaction to borax and the incidence of internal cork. Inasmuch as the symptoms of sweet-potato flesh degeneration associated with a deficiency of boron[4] were distinctly different from those of internal cork, the possibility that boron deficiency might be responsible for internal cork also was discarded.

In order to determine whether or not internal cork is transmitted from affected roots to a new crop produced by plants arising from them, several bushels of seed roots were cut in half and those with corky spots were selected for bedding in an isolated bed in the greenhouse. These seed pieces apparently produced sprouts in the normal manner. Later a duplicate plot was planted with vine cuttings taken from the original sprout-set plot. Similar plots were set adjacent to these with sprouts from an ordinary seed bed to serve as a check. All of the plots received the same fertilizer and cultural treatments. After harvest, samples were selected from each plot and exam-

[2] Personal communication.

[3] Walker, J. C. Internal black spot of garden beet. Phytopath. **29**: 120–128. 1939.

[4] Nusbaum, C. J. Internal brown spot, a boron deficiency disease of sweet potato. Phytopath. In press.

ined carefully for the incidence of internal cork. The disease was more than twice as prevalent in the crop originating from corky seed than from ordinary seed, as shown in table 1. Inasmuch as the internal cork status of the seed roots in the ordinary bed was not determined, these results are of doubtful significance. Nevertheless, suspicion is raised that the causal agent, whatever it may be, is root borne.

TABLE 1.—*Incidence of internal cork in Porto Rico sweet potatoes produced from sprouts and vine cuttings originating from ordinary and corky seed stocks, respectively*

Seed stock	Type of transplant	Total harvested roots exam.	Incidence of internal cork					
			None	1[a]	2[b]	3[c]	4[d]	Index[e]
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	
Ordinary	Sprouts	30	15	10	2	3	0	0.77
	Vine cuttings	30	12	12	3	1	2	0.97
Corky	Sprouts	30	3	8	8	5	6	2.10
	Vine cuttings	30	0	7	9	6	8	2.50

[a] Trace, only 1 spot per root.
[b] Slight, 2 to 5 spots per root.
[c] Weighted average of scale, none to 4, inclusive.
[d] Severe, spots numerous.
[e] Moderate, 5 to 10 spots per root.

Throughout the 1944 growing season sweet-potato plants, growing in various fields, were examined frequently in an attempt to discover any aboveground symptoms which could be associated with the production of internal cork affected roots. While the vines were growing vigorously through midseason, nothing of a suspicious nature was observed. Late in the season, however, when vine growth began to decline, the older leaves near the crown of many plants showed conspicuous purplish splotches, usually from about 0.5 to 3.0 cm. in diameter and scattered promiscuously over the ventral leaf surface. The areas were irregularly circular and were usually, but not always, in a ring design with green centers. Microscopic examination of the affected portions of leaves, after clearing them in lactophenol and staining with acid fuchsin, failed to reveal any disorganization of the leaf tissues. This symptom was generally associated with the development of corky roots but numerous exceptions were found. A possible correlation between the ring spot symptom and internal cork is indicated but has not been established.

DISCUSSION

Both the origin of internal cork of sweet potato and the exact extent of its occurrence are unknown. In South Carolina it is apparently widespread, but at a relatively low level. Thus far only one inquiry concerning the disease has been received by the writer. A farmer in Union County submitted typical specimens for diagnosis.

The problem of the etiology of internal cork remains unsolved. Although only a few of the possibilities have been investigated, the behavior of the

disease thus far suggests that a virus may be involved. Preliminary attempts to transmit the disease to apparently healthy sweet-potato plants and to various other crop plants is underway.

SUMMARY

A new disease of sweet potato, referred to as internal cork was recognized for the first time in the spring of 1944. The symptoms are described. The disease was widespread but at a relatively low level in South Carolina during the 1944 season. The results here presented indicate that neither a culturable pathogen nor boron deficiency are involved and that the causal agent, as yet undetermined, may possibly be transmitted by diseased roots. A probable correlation between the late season occurrence of a ring-spot symptom appearing on the older leaves of some plants and the development of corky roots was indicated but was not definitely established. The possibility that the disease may be caused by a virus is suggested. These results are given as a preliminary report while further experimental work is in progress.

EDISTO EXPERIMENT STATION,
BRANCH OF CLEMSON COLLEGE,
BLACKVILLE, S. C.

EFFECT OF STORAGE CONDITIONS ON SURVIVAL OF COLLETOTRICHUM GOSSYPII[1]

C. H. ARNDT

(Accepted for publication August 11, 1945)

In a study of the effect of storage conditions on the retention of viability by cotton seed the use of seed naturally infested by the anthracnose fungus, *Colletotrichum gossypii* South., afforded an opportunity to study coincidentally the survival of the infesting fungus. The results herein reported supplement the earlier data of Lehman (4) and Ludwig (5). The observed effect of the moisture content of the seed and temperature of storage should be of interest to individuals who may wish to preserve undiminished for some time the viability of fungi naturally infesting seeds. The storage conditions and their effect on the viability of the seed have been described by Simpson (6).

METHODS

For use in this storage test two lots of cotton seed naturally infested by the anthracnose fungus were obtained during the first week of October, 1937. One of the lots, Deltapine 11a, was procured from a gin at Pendleton, S. C., within 3 days after picking. The second lot, Carolinadel No. 2, was obtained from a gin at Westminster, S. C., within 10 days after picking. The initial moisture contents were 15.6 and 13 per cent, respectively. Each lot, after thorough mixing, was divided into five 100-lb. portions, and an attempt was then made to adjust these portions to moisture contents of 8, 10, 12, 14, and 16 per cent. These percentages will be used to designate the moisture contents, although the method of drying used gave actual moisture contents for the Carolinadel sub-lots of 7.2, 10.1, 12, 14.1, and 16.5 per cent; and for the Deltapine sub-lots of 7.7, 10.1, 12.1, 14, and 15.6 per cent. Additional moisture determinations were not made at the time of the tests reported in this paper. They were made, however, on 300-gm. samples which had been stored for 3 years in air-tight containers in a seed storage house at Clemson, S. C. The increases in moisture content of the samples with 7.2 to 12.1 per cent initial moisture content did not exceed 0.4 per cent. The moisture content of the sub-lots with initial contents of 14 and 14.1 per cent had increased to slightly above 15 per cent, and those at 15.6 and 16.5 per cent had increased to about 20 per cent.

To obtain moisture contents higher than the initial content, the requisite amount of water was sprinkled over the seeds, which were thoroughly mixed, then and again after 2 consecutive 24-hr. periods. In the interim and until

[1] Technical contribution No. 108 of the South Carolina Agricultural Experiment Station. Investigation made in cooperation with the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Acknowledgment is due G. W. Boozer, formerly agent of the above Division, for technical assistance in this study. This study was planned and carried out in cooperation with D. M. Simpson, U. S. Cotton Field Station, Knoxville, Tennessee.

placed in air-tight glass jars for storage, the seeds were kept in a moisture-proof container. Lower moisture contents were obtained by drying the seed in wire-bottomed trays in a drying room through which slightly heated air (not exceeding 36° C.) was circulated.

In order to obtain some information concerning the most convenient means of obtaining reproducible determinations of the moisture content of cotton seed, samples of the Carolinadel lot were dried in an electrically heated oven at 80°, 90°, and 100° C. At all 3 temperatures, there were comparable very small losses up to and including the 12th day; and the mean moisture contents of the 3 samples dried at each of the 3 temperatures, if calculated from the dry weights obtained at 80°, 90°, and 100°, would have been 11.8, 12.2, and 13.7 per cent, respectively. At 90°, there was relatively little change in weight after 3 days. About 5 days were required to attain a relatively constant weight at the other 2 temperatures. Since it was impossible to judge from these data which temperature and period of drying gave the most nearly correct indication of the moisture content, a temperature of 90° for 3 days was arbitrarily chosen for drying the seed on account of the ease of obtaining a relatively constant weight at this temperature. The moisture contents given by Simpson (6) are generally slightly lower than those given in this paper, since he dried his samples at 100° C. for 24 hours and used the initial seed weights (dry weight plus water) to calculate the moisture contents.

As soon as the moisture contents were adjusted, the seeds were placed in pint jars, which were sealed with rubber gaskets. They were then sent to Simpson (6), who placed seeds of the 5 moisture contents of both lots in storage December, 1937, at about 1°, 21°, 33° C., and the fluctuating temperatures of an unheated seed-storage house (in his paper referred to as "air temperature"), in which the seed were subjected to the range of the air temperature of Knoxville, Tenn., about −20° to 36° C. The data for this paper were obtained from samples removed from storage in December, 1938, May, 1939, and June, 1943.

To ascertain the survival of the anthracnose fungus, the seeds were germinated on water agar in test tubes, as described previously (1). A temperature of 23°–25° C. was maintained, since this is approximately the optimal temperature for the infection of cotton seedlings by this fungus (1). Emergence of the primary roots was well advanced after 3 days, and the cotyledons began to emerge from the testas after 5 days. The elongation of the hypocotyls usually made it necessary to remove the plugs on the 9th or 10th day. Final observations were made after 12 or 14 days of incubation. A seed was considered to have germinated only when a typical primary root and hypocotyl were formed. Seeds forming primary roots only were classified as non-germinating. Since Simpson (6) used the emergence of the radicle as a criterion of viability, the percentages of viable seeds reported by him are higher than those given in this paper for several sub-lots containing seeds which formed radicles but were unable to form normal seedlings.

The appearance of typical lesions on the hypocotyls or cotyledons was used as a criterion of the survival of the fungus without any implication as to whether the absence of lesions on the seedlings was due to the death of the mycelium of the fungus or a mere loss of its ability to infect the seedlings. All diseased seedlings with lesions atypical of those caused by *Colletotrichum gossypii* were examined microscopically to ascertain the cause of such lesions. Since no seedling inoculations were attempted to ascertain whether this fungus may have survived on the seeds which did not germinate, there are no comparable data for the survival of the fungus in sub-lots with few or no viable seeds at the time of sampling. The data on seed viability have been fully discussed by Simpson (6).

RESULTS

The effect of varying moisture content of the seed on the survival of the anthracnose fungus at a given temperature is well shown in figure 1 by the percentages of infected seedlings for the sub-lots stored for 5.5 years at 1° C.

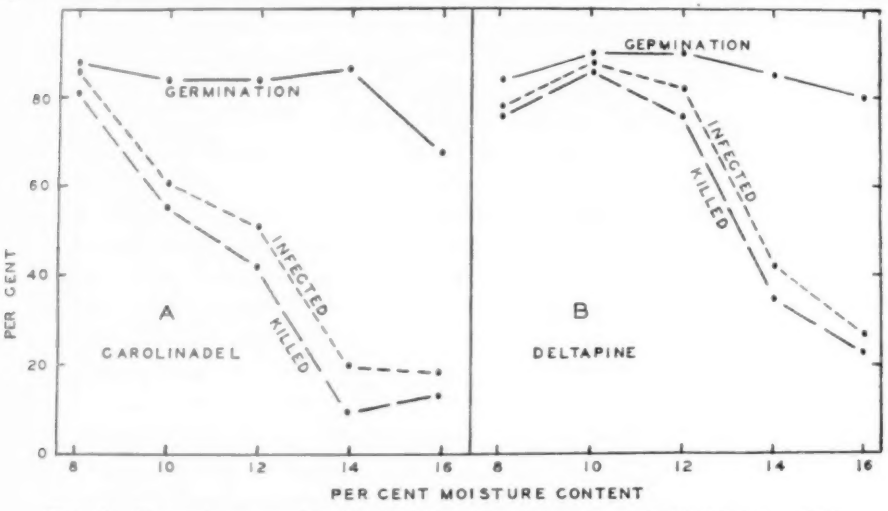

FIG. 1. Percentage germination of 2 lots of cotton seed of 8, 10, 12, 14, and 16 per cent moisture content after storage for 5.5 years at 1° C. Also number of seedlings infected and killed by the anthracnose fungus per 100 seed germinated.

For this temperature and storage period, more than 80 per cent of the seedlings of the Carolinadel sub-lot of 8 per cent moisture content and of the Deltapine sub-lots of 8, 10, and 12 per cent moisture content were infected. For the Carolinadel sub-lots, however, there were successive reductions in the number of seedlings infected and killed by the anthracnose fungus coincidentally with each increase in moisture content from 8 to 14 per cent. The Deltapine sub-lots showed a similar reduction only with the increase in moisture content from 12 to 14 per cent. The percentages of seedlings infected were about the same for the Carolinadel sub-lots of 14 and 16 per cent moisture content; but, for the corresponding Deltapine sub-lots a smaller percentage of the seedlings of the 16 per cent than of the 14 per cent moisture-content sub-lot were infected and killed.

At the higher storage temperatures of 21°, 33°, and air, the fungus infected relatively few seedlings after 17 months of storage at all 5 moisture contents. For the sub-lots which showed no loss of seed viability after this storage period, the highest percentage of infected seedlings was 20 for the Deltapine sub-lot of 8 per cent moisture content stored at 21° C. (Table 1). At air temperature, the highest infection was 14 per cent for the Carolinadel sub-lot of 12 per cent moisture content. Higher percentages of seedlings were infected for two other sub-lots, Carolinadel of 12 per cent moisture con-

TABLE 1.—*The effect of the moisture content and the temperature of storage of cotton seeds on their germination, the percentage of seedlings infected*[a] *by the anthracnose fungus, and the percentage of seedlings killed after incubation for 12 days at 24° C. Period of storage, 17 months*

Moisture content, per cent	Storage temperature	Carolinadel No. 2			Deltapine 11a		
		Germination, per cent	Seedlings		Germination, per cent	Seedlings	
			Infected, per cent	Dead, per cent		Infected, per cent	Dead, per cent
8	1° C.	90	83	71	97	100	93
	21°	93	6	6	88	20	7
	Air[b]	90	11	4	90	11	4
	33°	90	3	2	80	9	0
10	1°	90	89	78	89	90	71
	21°	86	5	0	90	2	0
	Air	90	6	2	85	6	5
	33°	20	0	0	42	0	0
12	1°	88	85	77	90	91	70
	21°	80	13	5	75	12	0
	Air	71	14	7	83	7	0
	33°	10	40	0	0	0	0
14	1°	86	91	80	85	88	71
	21°	36	8	8	50	4	4
	Air	0	0	0	12	33	0
	33°	0	0	0	0	0	0
16	1°	88	80	73	90	81	48
	21°	0	0	0	0	0	0
	Air	0	0	0	0	0	0
	33°	0	0	0	0	0	0

[a] Expressed as a percentage of the normal seedlings formed in the germination of a given sub-lot.

[b] In an unheated seed storage house at Knoxville, Tennessee.

tent stored at 33° and Deltapine of 14 per cent moisture content stored at air temperature; but these two sub-lots had only 10 and 12 per cent, respectively, viable seeds.

A reduction in the survival of the anthracnose fungus was also observed in germination tests made after 12 months of storage, when the percentages of infected seedlings were about the same as those given in table 1 for 17 months of storage. The percentages of infected seedlings after 12 months are, consequently, of interest only for two sub-lots of seed which showed a distinct loss of viability by the fungus between these two dates of sampling, the Carolinadel sub-lots of 8 per cent moisture content stored at 21° and air

temperature. After 12 months of storage, these two sub-lots had 51 and 37 per cent, respectively, of the seedlings infected; and after 17 months, 6 and 11 per cent, respectively. The losses in viability of the fungus were much less for the corresponding Deltapine sub-lots with the percentages infected after 12 months being 25 and 18, respectively, and after 17 months, 20 and 11, respectively. The generally higher survival of the anthracnose fungus in this study on seeds of relatively low moisture content and stored at a relatively low temperature coincides with the observations of Lehman (4) and Ludwig (5).

The data of table 1 also indicate that the retention of seed viability was favored by relatively low seed moisture contents and temperatures. After storage for 17 months, 1° C. was the only temperature at which the seeds of all 5 moisture contents showed no reduction in viability. Similarly, the seeds of 8 per cent moisture content were the only ones to retain their initial viability at all 4 temperatures. In addition, the seed of 10 per cent moisture content showed no loss of viability at 21° and air temperature, but those of 12 per cent showed a slight loss. Thus, the viability of both the seeds and the fungus was not greatly influenced by the moisture content of seeds for a storage period of 17 months at 1° C., but the survival of the fungus was greatly reduced during this period of storage at the other temperatures and a moisture content of the seed not exceeding 10 per cent is essential for the retention of the original seed viability at 21°. These results substantiate the earlier observations of Barre (2), Lehman (4), and Ludwig (5) to the effect that the viability of the anthracnose fungus naturally infesting cotton seeds under the usual storage conditions is generally less than that of the seeds. This difference in the relative rate of the loss of viability during storage between cotton seeds and the anthracnose fungus, lead to the recommendation that seed which had been stored for 1 to 3 years be used for planting (2, 3). The necessity for the use of such seed has been superseded by the development of effective seed treatments.

Additional data that indicated a better survival of the anthracnose fungus at relatively low temperatures and low seed moisture contents were obtained when isolating fungi from lesions on seedlings grown from seeds of these sub-lots removed from storage in 1941, or after 3.5 years, and planted in a field at Knoxville, Tenn. Sixty-nine seedlings which developed from seeds stored at 1° C. and which had lesions on the hypocotyls were surface sterilized and placed on non-nutrient agar. *Colletotrichum gossypii* was secured from 43 of these seedlings, and *Fusarium moniliforme* from 20. *C. gossypii* was not found on a single one of 76 seedlings representative of those developing from seeds stored at the other temperatures. In this field the mean seedling loss[2] for the seeds stored at 21°, 33°, and air temperature was about 4 per cent (maximal 13.3 per cent); while the losses for the Carolinadel sub-lots of 8 to 14 per cent moisture content stored at 1° ranged from 28 to 37 per cent, and those for the Deltapine from 45 to 62 per cent. The

[2] Personal communication, Mr. D. M. Simpson.

losses for the sub-lots of 16 per cent moisture content stored at the same temperature were only $\frac{1}{3}$ as large as those for the sub-lots of lower moisture contents. Comparable data, previously published by Simpson (6, table 6), for a field planting in 1940 (after $2\frac{1}{2}$ years of storage) indicate a similar adverse effect of a high seed moisture content on the survival of the anthracnose fungus.

Fusarium moniliforme was the predominating fungus on non-germinating seeds, while small percentages of seed were infected by *Aspergillus spp.*, a *Chaetomium sp.*, an *Ophiotrichum sp.*, and several other unidentified fungi. A *Rhizopus sp.* was present on the seeds of 14 and 16 per cent moisture content. All these fungi may have contaminated the samples after their removal from the storage containers. However, since they represent the typical fungus flora of non-germinating seeds, there is reason to believe that these species survived storage.

SUMMARY

Portions of 2 lots of cotton seed naturally infested by the anthracnose fungus, *Colletotrichum gossypii*, were adjusted to moisture contents of approximately 8, 10, 12, 14, and 16 per cent. Samples of each moisture content of each lot were placed in storage at 1°, 21°, and 33° C., and also at the air temperature of Knoxville, Tenn. After storage for 12, 17, and 66 months, seeds from these samples were germinated at 24° to ascertain the survival of the fungus.

The survival of the fungus, as indicated by the percentage of seedlings infected and killed, was undiminished after 12 and 17 months of storage on only the seeds stored at 1° C.

After storage for 5.5 years at 1° C., the fungus infected more than 75 per cent of the seedlings which developed from seeds stored at 8 per cent moisture content. A reduced number of seedlings tended to be infected with each successive increase in the seed moisture content up to 16 per cent, at which moisture content from 19 to 27 per cent were infected.

Under the storage conditions used, the anthracnose fungus infesting the seeds tended to lose its ability to infect seedlings before there was appreciable loss of viability by the seeds.

DEPARTMENT OF BOTANY,
AGRICULTURAL EXPERIMENT STATION,
CLEMSON, SOUTH CAROLINA.

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THE INTERNAL INFECTION OF COTTON SEED AND THE LOSS OF VIABILITY IN STORAGE[1]

C. H. ARNDT

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In earlier papers (1, 4) based on a cooperative study of cotton seed storage, the adverse effect of high storage temperatures and high seed-moisture contents on the longevity of cotton seed and the anthracnose fungus infesting such seed was noted. The results left unanswered the question as to whether loss of viability of the seeds in storage was due primarily to autocatalytic changes within the seeds or to infection of the seeds by micro-organisms. Both of these processes should have been favored by the higher moisture contents and by the higher storage temperatures used. The observations reported in this paper were made to ascertain whether micro-organisms were generally present in the interior of the seeds at the beginning of seed deterioration, since their presence or absence should indicate the relative rôles of infection and autocatalytic changes.

METHODS

Cotton seed from 2 storage tests were used. In one test (1, 4) made under controlled conditions, portions of 2 lots of seed, Carolinadel and Deltapine, were adjusted to approximately 8, 10, 12, 14, and 16 per cent moisture content and were then stored at 1°, 21°, 33° C., and at the air temperature of Knoxville, Tenn. The other seeds were obtained from a cooperative locality-of-storage test, supervised by D. M. Simpson, in which portions of a lot of Stoneville–2b seed were stored in ordinary seed houses at 7 localities in the southern United States, as listed in table 1. One-half of each original lot of seed was acid-delinted, and the 4 bags of seed stored at each locality were: (a) fuzzy seed not treated, (b) fuzzy seed treated with 2 per cent Ceresan, (c) acid-delinted seed, and (d) acid-delinted seed treated with 2 per cent Ceresan.

If germination tests made by Simpson indicated loss of viability by a given sub-lot of seed, samples were sent the writer. Individual seeds were cultured in test tubes on sterile water agar at 24° C. (1). In order to remove all external contamination by micro-organisms before culturing, the seeds not previously delinted were acid-delinted, washed in tap water, and air-dried. Immediately before they were placed in the test tubes, all seeds were immersed for 2 minutes in a solution of $HgCl_2$ in 50 per cent ethanol (2.5 gm./l.), and were then washed with sterile water. One hundred seeds were cultured for each sample and were observed for at least 2 weeks.

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The development of a seed into a seedling with a normal primary root, hypocotyl, and cotyledons was used as a criterion of normal germination and correspondingly of a normal viable seed. The loss of viability by the seeds was associated with the appearance of abnormal seedlings which have been grouped into 2 classes to facilitate the presentation of the data: (a) those

TABLE 1.—*Germination of a lot of Stoneville–2b cotton seed after storage for 20 months in 7 different localities. Data are based on the germination of 100 seeds*

Location	Kind of seed[a]	Kinds of seedlings from viable seeds				Nonviable seeds	
		Normal	Normal tops, abnormal radicles	No tops, abnormal radicles			
				Total	Infected[b]	Total	Infected[b]
Clemson, S. C.	FU	86	2	12		0	
	FT	62	30	5		3	
	DU	88	0	12		0	
	DT	70	0	17		13	
Jackson, Tenn.	FU	85	0	8	6–Pen.	7	2–Bact.
	FT	70	0	22		8	
	DU	60	2	20	2–Asp.	18	
	DT	63	2	35		0	
Knoxville, Tenn.	FU	70	0	17	2–Fus.	13	8–Bact.
	FT	72	0	12		16	3–Bact.
	DU	70	0	12		18	5–Bact.
	DT	55	10	12		23	8–Bact.
Greenville, Texas	FU	88	2	0		10	
	FT	60	0	27		13	3–Bact.
	DU	57	3	30		10	
	DT	55	5	40		0	
State College, Miss.	FU	50	17	22		11	2–Bact.
	FT	50	5	40		5	
	DU	65	2	28	2–Asp.	5	2–Pen.
	DT	10	5	47		38	8–Bact.
Florence, S. C.	FU	20	22	24		34	10–Bact.
	FT	38	10	35		17	3–Pen.
	DU	8	2	32		58	12–Bact.
	DT	30	5	50		15	
Baton Rouge, La.	FU	8	5	65		22	2–F., 3–Bact.
	FT	5	10	53		32	
	DU	10	5	33		52	10–Pen., 2–Asp., 8–Bact.
	DT	2	5	43		50	2–Bact.

[a] FU, fuzzy seed not treated; FT, fuzzy seed treated with Ceresan; DU, acid-delinted seed, not treated; DT, acid-delinted seed treated with Ceresan.

[b] Abbreviations following the numbers refer to species of *Penicillium*, *Aspergillus*, *Fusarium*, to a nonsporulating fungus (F.), or to bacteria.

that formed short radicles but no evident hypocotyls and whose cotyledons did not emerge from the testas; and (b) those that formed normal hypocotyls and cotyledons but did not form primary roots of normal length. The abnormal primary roots were usually from 3 to 6 cm. long, and their apices were blunt and dark. When normal cotyledons developed on such seedlings

numerous secondary roots were generally formed at the upper portion of the radicle and the base of the hypocotyl, which permitted the continued growth of the seedlings. Total germination is used to designate the percentage of seeds that formed radicles, regardless of the normality of subsequent development, and is equivalent to the ordinary usage of this term in germination tests. The various kinds of abnormal seedlings produced by the sub-lots, after a loss of viability was evident, are illustrated in figure 1.

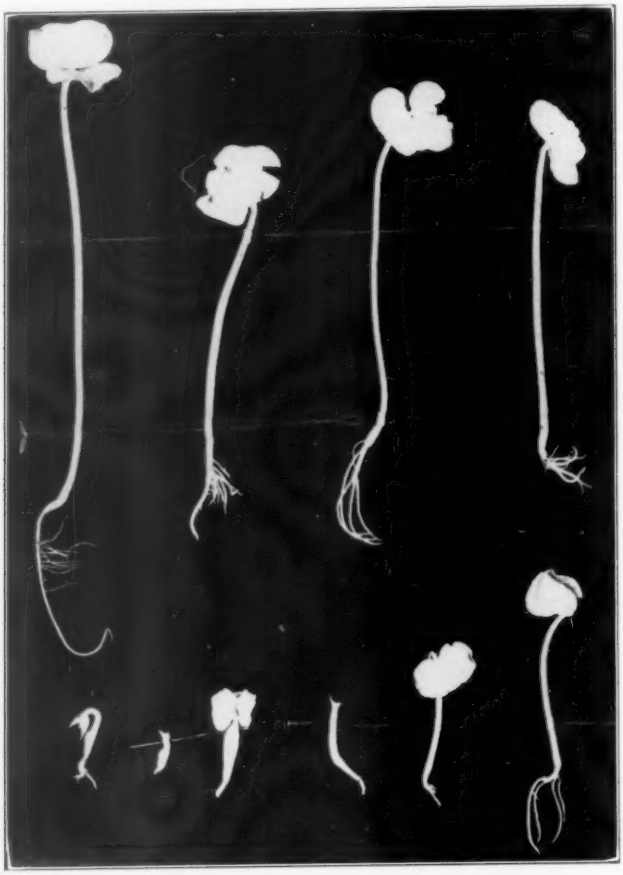

FIG. 1. Abnormal seedlings produced by cotton seed of 12 per cent moisture content after storage for 28 months in a seed house at Knoxville, Tenn. Seedlings photographed after 10 days' incubation at 24° C. Normal seedling, upper left.

RESULTS

There were large differences in the rate of seed deterioration among the 7 localities in which the Stoneville lot was stored (Table 1). The conditions that may have been responsible for these differences will be discussed by Simpson in a later paper. The differences in rates of deterioration among the sub-lots of seed of several moisture contents stored at different temperatures were clearly related to both moisture content and temperature. Via-

bility was lost more rapidly, in 4 months, in the lots of 16 per cent moisture content stored at 33° C. (Table 2); while for seed of 8 per cent moisture content stored at the same temperature, the viability was undiminished after 5.5 years (1). The data of table 2 indicate an increase in the rate of deterioration with each successive increase in the moisture content and temperature for lots which are otherwise comparable. One sub-lot, B–10–33 of table

TABLE 2.—*Percentage germination and types of seedlings obtained from two lots of seed of five different moisture contents after storage at different temperatures*

Variety, moisture content, temperature, and length of storage[a]	Germination and types of seedlings[b]			
	Total	Normal seedlings	Normal tops, abnormal radicles	No tops, abnormal radicles
A–16–33–4	0	0	0	0
B–16–33–4	0	0	0	0
A–14–33–4	65	28	18	19
B–14–33–4	1	0	0	1
A–12–33–4	89	74	7	8
A–12–33–12	50	29	9	12
B–12–33–4	81	50	10	21
B–12–33–12	54	28	7	19
B–12–33–17	0	0	0	0
A–10–33–12	87	87	0	0
A–10–33–17	72	16	18	38
B–10–33–12	60	45	9	6
B–10–33–17	74	5	20	49
A–16–air–12	0	0	0	0
B–16–air–12	0	0	0	0
A–14–air–12	39	23	8	8
A–14–air–17	0	0	0	0
B–14–air–12	48	26	19	3
B–14–air–17	18	0	0	18
B–14–air–28	0	0	0	0
A–12–air–28	45	8	17	20
B–12–air–28	47	14	13	20
A–16–21–12	0	0	0	0
B–16–21–12	35	15	12	8
A–14–21–17	26	0	0	26
A–14–21–28	0	0	0	0
B–14–21–17	71	50	0	21
B–14–21–28	41	3	8	30
B–12–21–17	95	89	0	6
A–12–1–28	88	86	2	0
B–12–1–28	90	87	0	3

[a] Variety A is Carolinadel; variety B is Deltapine. A–16–33–4 designates a seed lot of Carolinadel of 16 per cent moisture content stored at 33° C. for 4 months. "Air" refers to the temperature of a seed house at Knoxville, Tenn.

[b] Based on 100 seeds.

2, shows an apparently higher germination after 17 than after 12 months of storage, but the percentage of normal seedlings was smaller after the longer storage period. When there was little or no loss of viability, the percentages of normal seedlings for the sub-lots of table 2 approximate the percentages of germination based on radicle protrusion reported by Simpson (4); but, after deterioration was evident, the percentages reported by Simpson approximate the sum of the normal and abnormal seedlings of table 2.

The concurrent increase in the percentage of abnormally germinating seeds accompanying a loss of viability may be indicated by grouping the sub-lots of table 2 into 4 groups with the total percentage of viable seeds (a) more than 80, (b) between 60 and 80, (c) between 40 and 60, and (d) less than 40. For these 4 groups, the percentages of normal seedlings were 90, 42, 38, and 32, respectively; the percentages of embryos that formed tops but had abnormal radicles were 3, 19, 26, and 17, respectively; and the percentages of embryos that formed only short radicles were 7, 39, 36, and 51, respectively. The same increase in the percentage of abnormal seedlings was also shown by individual sub-lots. Thus, for sub-lot A–10–33 during the interval between sampling after 12 and 17 months, the total germination decreased from 87 to 72 per cent; while the percentage of seedlings with abnormal roots increased from 0 to 18 and the percentage which formed only radicles increased from 0 to 38.

The small percentages of seedlings infected by micro-organisms indicated that infection of the seeds was not the primary cause of the loss of viability. Less than 2 per cent of the seedlings obtained in the germination of seeds from the controlled-condition storage test were infected; and these were all seedlings with dark, blunt apices, which were infected by a *Penicillium sp.* In the locality-of-storage test only 12 seedlings were infected, and these were abnormal seedlings with short radicles and without hypocotyls and cotyledons that had developed from seeds that had not been treated with Ceresan (Table 1).

The percentages of nonviable seeds which were internally infected were much higher than the percentages for the viable seeds, but generally not all were infected, as shown in table 3 for sub-lots A–10–33–12, A–10–33–17, B–10–33–12, A–14–air–12, and B–14–21–17. Four of the sub-lots with no viable seed had practically all seeds infected; while a fifth lot, A–16–21–12, had only 68 per cent infected. The percentages of infected nonviable seeds were generally smaller in the locality-of-storage test than in the controlled-condition test, only 19 per cent of the 491 nonviable seeds of the former being infected (Table 1). The treatment of the seeds with Ceresan before storage did not prevent the infection of these seeds by fungi and bacteria, although a smaller percentage of the treated than of the untreated seeds was infected.

The Deltapine sub-lot of 14 per cent moisture content stored at 21° C. illustrates the increase in percentage of nonviable seeds infected with an increased length of storage. After 17 months of storage only 7 per cent of the seed were infected (no infection of viable seeds), but after an additional 11 months of storage 50 per cent were infected. Similar increases are evident for the sub-lots A–12–33, A–14–air, B–14–air, and A–14–21.

The predominating fungus obtained from the infected nonviable seeds was a white-spore species of *Aspergillus*. Yellow- and black-spore species of this genus were less abundant. The genus *Penicillium* was represented by several species; a grayish-spore species was found most frequently, with yellow- and green-spore species found less frequently. As indicated in table

3, *Diplodia theobromae* was found only in the Carolinadel sub-lot of 16 per cent moisture content after 4 months storage at 33° C., when 40 per cent were infected. This lot of seed was infested externally by this fungus at the start of the experiment. Its failure to infect the nonviable seeds of the other sub-lots indicates that a relatively high temperature and a high moisture

TABLE 3.—*Percentage of non-viable seeds for the two lots of cotton seed listed in table 2 and the number of seeds per 100 seeds cultured that were infected by several species of fungi and bacteria*

Variety, moisture content, temperature, and length of storage[a]	Percentage non-viable seed	Number of seeds infected by micro-organisms				
		Total	Bacteria	*Aspergillus spp.*	*Penicillium spp.*	Other fungi[b]
A–16–33–4	100	100	10	20	30	40–Dip.
B–16–33–4	100	96	0	93	3	0
A–14–33–4	35	19	3	3	12	1–Col., 1–F.
B–14–33–4	99	96	0	88	8	0
A–12–33–4	11	7	1	0	5	1–F.
A–12–33–12	50	28	6	12	4	6–F.
B–12–33–4	19	6	0	0	4	2–F.
B–12–33–12	46	20	6	4	6	4–F.
B–12–33–17	100	33	33	0	0	0
A–10–33–12	13	0	0	0	0	0
A–10–33–17	28	10	10	0	0	0
B–10–33–12	40	14	6	2	4	2–F.
B–10–33–17	26	16	11	2	1	2–F.
A–16–air–12	100	68	54	8	6	0
B–16–air–12	100	66	46	12	6	0
A–14–air–12	61	22	16	0	2	2–Col., 2–F.
A–14–air–17	100	80	80	0	0	0
B–14–air–12	52	22	20	2	0	2–Col.
B–14–air–17	82	50	50	0	0	0
B–14–air–28	100	94	94	0	0	0
A–12–air–28	55	49	49	0	0	0
B–12–air–28	53	53	53	0	0	0
A–16–21–12	100	68	54	8	6	0
B–16–21–12	65	42	20	22	0	0
A–14–21–17	74	45	45	0	0	0
A–14–21–28	100	97	97	0	0	0
B–14–21–17	29	7	7	0	0	0
B–14–21–28	59	52	50	0	0	0
B–12–21–17	5	4	2	0	0	0
A–12–1–28	12	7	7	0	0	1–Col.
B–12–1–28	10	8	6	0	0	2–Fus.

[a] See footnote to table 2.

[b] Abbreviations following the numbers refer to *Diplodia theobromae, Colletotrichum gossypii,* species of *Fusarium,* or to a nonsporulating fungus (F.).

content are essential for the infection of the seed by this fungus. When first stored (1) both of these lots of seed were also infested by several species of *Fusarium* and by *Colletotrichum gossypii.* The results (Table 3) show that the latter fungus was obtained from only 6 seeds after deterioration of the seed had started; and Fusaria were obtained from only 2 seeds. Apparently, infection of seeds by these fungi did not increase after storage or they were overgrown by various saprophytes. The seeds which were infected by fungi

also were invariably infected by several species of bacteria, of which the most common was a species which formed white colonies on agar. This same species was also the most prevalent of the bacteria which infected the seeds when they were not infected by fungi.

DISCUSSION

The failure to demonstrate infection, by micro-organisms, of most of the abnormal seedlings and many of the nonviable seeds seems to indicate that the initial loss of viability was probably associated with autocatalytic processes within the embryo itself, as described by Katon and Altschul (2). Since the meristem of the primary root was the first to lose its capacity for indefinite growth, this portion of the embryo is apparently more susceptible to injury by such processes than the other meristems. Similar injury has been noted in other studies in which seeds that had been stored in the laboratory were germinated in sand culture. The first evidence of reduced viability was delayed emergence of a portion of the seedlings. The apices of the radicles of these seedlings were invariably blunt and dark. These seedlings, in part, formed secondary roots at the base of the hypocotyl and upper portion of the primary root (Fig. 1), and they developed into normal plants. The more severely affected primary roots grew in almost any direction (at times even emerging from the sand) as if they had lost their capacity for normal geotropic response.

Although the data indicate that internal infection of the seeds by bacteria and fungi could not have been the primary cause of the loss of viability of the seeds under the storage conditions of these studies, this does not apply necessarily to the sub-lots of 14 and 16 per cent moisture content stored at 33° C. which lost their viability during the first 4 months of storage, and which were almost 100 per cent infected at the time of the first examination.

The technique used in these studies did not eliminate the possibility that in certain instances the loss of viability may have been accelerated by the action of fungi and bacteria on the peripheral portions of the seed, if it may be assumed that the surface treatment of the seeds with H_2SO_4 and the $HgCl_2$ solution killed or inhibited the growth of such fungi and bacteria, when the seeds were cultured on water agar. This possibility seems to be definitely eliminated by the fact that the deterioration of the Ceresan-treated sub-lots in the locality-of-storage test was not greatly different from that of the untreated seed. Similarly, Robertson *et al.* (3) found that there was little difference between the rate of deterioration of untreated and Ceresan-treated barley, oats, and wheat seed which had been stored at several relative humidities.

SUMMARY

Cotton seed in which there was a reduction of viability after storage were surface-sterilized and germinated under sterile conditions. In one study, seeds of 5 moisture contents were stored at 4 temperatures (1°, 21°, 33° C., and that of Knoxville, Tennessee) for periods of 4, 12, 17, and 28 months. In another study, seeds were stored in 7 localities in ordinary storage houses.

A negligible percentage of the viable seeds, including those that formed abnormal seedlings, were infected by bacteria or fungi, and many of the non-viable seeds were not infected, which indicates that micro-organisms are not necessarily the primary cause of the deterioration of cotton seed at ordinary storage temperatures when the initial moisture content of the seed is 14 per cent or less.

The onset of the loss of viability by the seeds was associated with the appearance of abnormal seedlings. The various abnormalities indicated that the meristem of the primary root was the first portion of the embryo to be injured during storage and to lose its capacity for indefinite growth, although it generally retained its capacity for limited growth longer than the meristem of the hypocotyl. The primary roots of abnormal seedlings rarely developed beyond a length of 4 to 6 cm.; and their apices were generally blunt and discolored.

DEPARTMENT OF BOTANY,
AGRICULTURAL EXPERIMENT STATION,
CLEMSON COLLEGE, SOUTH CAROLINA.

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SOME FACTORS INFLUENCING CURLY TOP VIRUS CONCENTRATION IN SUGAR BEETS

N. J. GIDDINGS[1]

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INTRODUCTION

In earlier studies on susceptibility of sugar beet to the curly-top disease (1) there was evidence suggesting that the virus concentration in infected resistant beets used as virus sources was lower than in infected plants of the more susceptible varieties. It often happened that an appreciably lower percentage of test plants became infected when the vectors were leafhoppers that had fed on diseased, resistant plants than when similar vectors had fed on diseased, susceptible plants. This was not given much consideration at that time because it was recognized that there were many other factors which influence the amount of infection among test plants. As highly resistant varieties of sugar beet were developed it appeared more important to determine the relationship between resistance and virus concentration.

In 1929, Holmes (5) reported a convenient and accurate method of determining the relative concentrations of tobacco mosaic virus, but no similar "local lesion" method for use with the curly-top virus, *Ruga verrucosa* Carsner and Bennett, has been discovered. Lackey (6) reported that root tips of curly-top infected sugar beets and of beans had a higher virus concentration in the portion below the protophloem sieve tubes than in portions just above that region. Using virus strain 1, he reported (7) that the root tips of resistant sugar beets had a higher virus concentration than those of the susceptible beet. He also reported (8) that root tips of the susceptible bean variety Bountiful had a higher virus concentration when infected with the more virulent strain 1 than when infected with the less virulent strain 4, but that in root tips of susceptible beets the less virulent strain 4 reached a somewhat greater concentration than strain 1. In all of these experiments he fed nonviruliferous leafhoppers on juice from the root tips to be tested and then caged them on young, susceptible sugar-beet plants, using one leafhopper to a plant.

METHODS

Nonviruliferous leafhoppers were fed for a short period upon plants infected with a known virus strain and hereafter designated as "source plants." They were then transferred to healthy, young sugar-beet plants, one leafhopper being caged on each test plant. The percentage of plants which became infected from each source plant was determined and it was assumed that such infection data gave good evidence as to relative concentrations of curly-top virus.

[1] Senior Pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

Young adult leafhoppers have been more satisfactory than nymphs or old adults. Nymphs were sometimes found moulting in the cages on source plants and at this stage they may not feed for some hours. The older adults were likely to feed less than active nymphs or young adults and to die in greater numbers than the young adults.

The sugar-beet varieties used as source plants were the highly resistant S.L. 68 and the very susceptible S.L. 842. The curly-top virus strains used were those previously described (2) as strain 1 and strain 2. Each of these strains readily infects the resistant beet but neither of them induces severe injury in this host. Plants of the resistant S.L. 68 infected by strain 1 show vein clearing, occasional small papillae along the veins on the underside of the leaves and in many cases some rolling of the leaves (See (1R) in figure 1). Plants of S.L. 68 infected by strain 2 show similar but less obvious

FIG. 1. Typical curly top symptoms on young sugar beets 30 days after inoculation; beet variety S.L. 842 infected by virus strain 1 (1), beet variety S.L. 68 infected by virus strain 1 (1R), and beet variety 842 infected by virus strain 2 (2).

symptoms. It is often necessary to look rather closely at plants of this resistant variety infected with strain 2 to detect any symptoms. Plants of the susceptible variety S.L. 842 are readily infected by either virus strain. In such plants infection by strain 1 causes extreme dwarfing and distortion of the foliage, while infection by strain 2 induces relatively little distortion or injury (Fig. 1). Symptoms induced in plants of the susceptible variety by strain 2 were similar to those induced in plants of the resistant variety by strain 1. The source plants selected for use were as nearly comparable as practicable but differences in severity of symptoms were unavoidable.

Feeding periods of nonviruliferous leafhoppers on source plants were varied from 15 minutes to 72 hours. For feeding periods of ten hours or less the leafhoppers were caged on the source plants during the day. In the earlier experiments the source plants were kept in an auxiliary greenhouse where the temperature range might be anywhere from 70° to 100° F. A high temperature and a relatively short feeding period on the source plants appeared to give better results than longer feeding periods at lower temperatures, so the last seven experiments were conducted with the source plants in a special feeding chamber where it was possible to maintain a

temperature of about 100° F. A feeding period of two or three hours was found adequate under these conditions.

Leafhoppers which had fed on the source plant to be tested for virus concentration were transferred to young sugar beet plants in the early two-leaf stage and permitted to feed on these for a week at ordinary greenhouse temperatures ranging from 60° to 90° F. Each test plant was inoculated through the cotyledon, using a small leaf cage and one leafhopper to a plant.

The curly-top susceptible beet S.L. 842 was used as a test plant in every experiment and the highly resistant beet S.L. 68 was also used as a test plant in a number of experiments. All test plants were set out in boxes $22\frac{1}{4}$ inches long by $5\frac{1}{2}$ inches wide by $4\frac{3}{4}$ inches deep, inside dimensions, with 10 or 12 plants arranged as five or six pairs lengthwise of the box. Inoculations were made in pairs so that each virus source occurred on plants adjacent to two other sources which were to be compared with it and so that inoculations from the same source did not always occur at the same position in the box. This gave something approaching randomized arrangement of the test plants with different virus sources and assured comparable results.

EXPERIMENTAL WORK

The method used for determining virus concentration cannot be considered very exact. In view of this, and since there is abundant evidence of differences in virus concentration of individual source plants, it seems best not to include detailed data covering each of the experiments.

Report on a Typical Experiment. The detailed data from a representative experiment are given in table 1. This table gives data to indicate relative concentration of curly-top virus in resistant source plants and in susceptible source plants. Plants 1 and 2 are considered as one pair of source plants, 3 and 4 as another pair, and so on. The concentration of virus strain 1 was tested from two pairs of source plants and for three periods of feeding, making six paired tests. The concentration of virus strain 2 was tested from three pairs of source plants and for three periods of feeding, making nine paired tests. Each of these tests was planned to include 24 plants and variation from that number was due to death of an occasional plant. This experiment included the inoculation of 714 test plants from 10 source plants.

Relative virus concentrations in plants infected by strain 1 and in those infected by strain 2 are revealed in table 1 by considering plants 1 and 5 as one pair, 3 and 7 as another, 2 and 6 as another, and 4 and 8 as another. Plants 9 and 10 cannot be considered for strain comparison as they were each infected with strain 2. The data for this comparison were secured from 571 test plants and from 8 source plants. In this case some data for comparing concentrations of strains 1 and 2 were secured incidentally from an experiment set up primarily to determine possible virus concentration differences between the resistant and the susceptible source plants. Many experiments were set up for the single purpose of comparing resistant source plants and susceptible source plants. Other experiments involved only

TABLE 1.—*Typical summary sheet from curly-top virus concentration experiments. Data from tests of March 3, 1939, including 15 tests which cover 5 pairs of source plants, 3 periods of feeding, and 2 strains of virus. Tests made upon young plants of susceptible sugar-beet variety S.L. 842*

Leafhopper feeding period on source plants	Virus from susceptible source plants					Virus from resistant source plants				
	Source plant number[a]	No. of plants inoc.	Plants diseased		Average incubation period	Source plant number[a]	No. of plants inoc.	Plants diseased		Average incubation period
			Number	*Per cent*	*Days*			*Number*	*Per cent*	*Days*
3 hrs.	1	23	12	52	9.3	2	24	8	33	9.1
	3	24	15	63	8.8	4	24	13	54	11.2
	5	23	12	52	10.1	6	24	7	29	10.1
	7	24	4	17	10.5	8	24	11	46	11.1
	9	24	13	54	12.2	10	24	7	29	11.4
6 hrs.	1	24	18	75	9.4	2	23	14	61	9.7
	3	24	16	67	9.3	4	24	17	71	9.7
	5	23	17	74	10.1	6	24	11	46	13.4
	7	24	10	42	12.3	8	24	13	54	11.2
	9	24	13	54	9.3	10	24	8	33	11.6
9 hrs.	1	24	18	75	9.3	2	24	14	58	11.1
	3	24	19	79	9.7	4	24	15	63	9.8
	5	24	21	88	10.0	6	23	16	70	11.1
	7	24	11	46	11.6	8	24	15	63	12.9
	9	24	18	75	10.2	10	23	12	52	12.8

[a] Source plants 1 through 4 had been inoculated January 9, 1938, with virus strain 1. Source plants 5 through 10 had been inoculated January 9, 1939, with virus strain 2.

comparisons of source plants infected by strain 1 with plants infected by strain 2.

Individual variation in the source plants is indicated by plant 7 in table 1 which showed the lowest virus concentration of any of the susceptible source plants for each of the three feeding periods. Plant 10 gave similar evidence as a resistant source. Plant 7 happened to be paired against a resistant source plant, No. 8, which consistently showed a relatively high virus concentration for strain 2 so that in each of the three feeding periods the resistant source plant indicated a higher concentration than the susceptible.

The data in table 1 also indicate that plants infected by virus strain 1 contained a higher concentration of virus than those infected by strain 2. This was true in both the resistant group of source plants and the susceptible group. Both the percentages of plants infected and the periods of incubation suggest a greater virus concentration for strain 1. Another possibility would be that the longer incubation period for strain 2 is due to a slower response on the part of the host. The incubation period was not used as a factor in determining the significance of data dealing with relative concentrations.

The six paired tests involving strain 1 show only one case, plants No. 3 and 4 in the six hour period, in which the resistant source plant indicated a higher virus concentration than the susceptible. In five of these six tests the incubation period was longer for plants infected from the resistant source plants than for those infected from the susceptible source plants. The nine paired tests involving strain 2 showed three instances in which the resistant source plant indicated a higher virus concentration than the susceptible and the same pair of source plants, 7 and 8, were involved in each case. The susceptible source plant carried a higher virus concentration in the other six tests. Such data from one experiment do not indicate significant differences, but similar results from many experiments were highly significant when subjected to the χ^2 test for significance.

Relative Concentration as Influenced by Resistance of Beet. The highly resistant sugar beet variety S.L. 68 and the susceptible variety S.L. 842 were used as virus-source plants. The tests were conducted with curly-top virus strains 1 and 2. Each of these strains readily infects either the susceptible or the resistant sugar beet but strain 1 induces relatively much more severe symptoms than strain 2.

The tests on susceptible plants from source plants carrying strain 1 showed that the susceptible source plants contained a higher virus concentration than the resistant source plants in a very large percentage of the tests (Table 2). On susceptible test plants there was 11 per cent more infection among the total of 1,673 inoculated with strain 1 from susceptible source plants than among the total of 1,667 inoculated from resistant source plants (Table 3).

Tests on resistant plants from source plants carrying strain 1 gave a still larger percentage of tests in which the susceptible source plants showed the

TABLE 2.—*Consolidated data from tests to determine the relative curly-top virus concentrations in resistant and in susceptible sugar-beet plants*[a]

Source of virus and susceptibility of test plants	Source plant pairs[b]	Feeding periods	Total tests	Tests showing					
				No difference in virus concentration		Lower virus concentration in susceptible source plant		Lower virus concentration in resistant source plant	
	Number	*Number*	*Number*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Source plants infected with strain 1 virus									
Test plants susceptible	35	27	74	5	7	15	20	54	73
Test plants resistant	13	9	39	2	5	7	18	30	77
Source plants infected with strain 2 virus									
Test plants susceptible	8	9	24	0	0	4	17	20	83
Test plants resistant	3	3	9	0	0	0	0	9	100

[a] Resistant source plants were the sugar-beet variety S.L. 68 and the susceptible source plants were the variety S.L. 842.

[b] Each pair of source plants consisted of one resistant sugar beet and one susceptible sugar beet, each infected with the same strain of virus and otherwise comparable.

higher virus concentration (Table 2). On resistant test plants there was 11 per cent more infection among the total of 890 inoculated from susceptible source plants than among the total of 893 inoculated from resistant source plants (Table 3).

This 11 per cent difference between the amount of infection from susceptible source plants and the amount from resistant source plants is the same as the difference shown when susceptible source plants were used but the eleven point increase over 23 per cent in the case of resistant test plants is nearly one-half, whereas the same increase over 54 per cent in the case of susceptible test plants is only one-fifth. The infection percentages secured from resistant source plants and from susceptible source plants show very different ratios depending upon whether the test plants are resistant or susceptible. Similar differences are evident in all experiments involving the resistant and the susceptible source plants.

TABLE 3.—*Summary of results of test-plant inoculations made from resistant and from susceptible sugar-beet plants during studies to determine relative curly-top virus concentrations*[a]

Source and strain of virus	Resistant test plants			Susceptible test plants		
	Inoculated	Diseased		Inoculated	Diseased	
	Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
Susceptible plants carrying strain 1	890	305	34	1673	1085	65
Resistant plants carrying strain 1	893	202	23	1667	905	54
Susceptible plants carrying strain 2	564	331	59	565	407	72
Resistant plants carrying strain 2	215	26	12	213	69	32

[a] The resistant sugar beet was the variety S.L. 68 and the susceptible beet was the variety S.L. 842.

Comparative tests for virus concentration in the susceptible beet and in the resistant beet when infected by virus strain 1 are somewhat complicated by the fact that the symptoms are different in the two beet varieties. The susceptible beet is greatly dwarfed and distorted whereas the resistant beet is not dwarfed and has relatively mild symptoms (Fig. 1). The extreme dwarfing of the susceptible plants means that the available tissue for leafhoppers to feed upon is much less than in the case of an infected, resistant plant of the same age. This might mean less favorable feeding conditions due to crowding of the leafhoppers.

Susceptible sugar beet plants infected with curly-top virus strain 2 do not show the dwarfing and distortion mentioned in connection with strain 1 and so might be thought to give a more accurate picture as to the relative concentrations. Tests on susceptible plants from source plants carrying strain 2 give a still greater percentage in which the susceptible source plant carried a higher virus concentration than the resistant source plant (Table 2). Of the total susceptible test plants inoculated there was 40 per cent more infection among those inoculated from susceptible source plants than among those inoculated from the resistant source plants (Table 3).

TABLE 4.—*Consolidated data from tests to determine the relative curly-top-virus concentrations in plants infected by strain 1 and in plants infected by strain 2*[a]

Source of virus and susceptibility of test plants	Source plant pairs[b]	Feeding periods	Total tests	Tests showing					
				No difference in virus concentration		Lower virus concentration for strain 1		Lower virus concentration for strain 2	
	Number	*Number*	*Number*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Susceptible source plants									
Susceptible test plants	21	9	61	5	8	17	28	39	64
Resistant test plants	17	9	47	3	6	8	17	36	77
Resistant source plants									
Susceptible test plants	10	9	30	2	7	5	17	23	77
Resistant test plants	6	6	18	0	0	0	0	18	100

[a] According to the amount of injury induced on susceptible and on resistant sugar beets, strain 1 is highly virulent while strain 2 has relatively low virulence.

[b] Each pair of source plants consisted of one sugar beet infected with strain 1 and one infected with strain 2. The two plants were of the same sugar-beet variety and otherwise comparable.

Tests on resistant plants from source plants carrying strain 2 showed a higher virus concentration for the susceptible source plant in every instance (Table 2). Of the total resistant test plants inoculated there was 47 per cent more infection among those inoculated from susceptible source plants than among those inoculated from the resistant source plants (Table 3).

The difference in virus concentration was indicated more strikingly when resistant test plants were used than when susceptible test plants were used. This was true whether considered on the basis of tests made or on the actual number of test plants inoculated and for both virus strains 1 and 2. It is also evident that the difference in concentration was greater, or that the

TABLE 5.—*Summary of results of test-plant inoculations made from sugar-beet plants infected with strain 1 or with strain 2 during studies to determine relative curly-top-virus concentration*[a]

Source and strain of virus	Resistant test plants			Susceptible test plants		
	Inoculated	Diseased		Inoculated	Diseased	
	Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
Strain 1 from susceptible plants	958	242	25	1282	661	52
Strain 2 from susceptible plants	948	100	11	1282	530	41
Strain 1 from resistant plants	372	65	17	664	358	54
Strain 2 from resistant plants	379	20	5	664	267	40

[a] According to the amount of injury induced on susceptible and on resistant sugar beets, strain 1 is highly virulent while strain 2 has relatively low virulence. Data in this summary are not comparable with data in table 3. The data for strain 1 virus from susceptible source plants on resistant test plants are typical of each group. The leafhopper vectors used on 10 per cent of the 958 plants (Table 5) had fed for 6 hours to 48 hours on source plants and 90 per cent of them had fed for 3 hours or less on source plants, whereas the vectors used on 61 per cent of the 890 plants (Table 3) had fed for 6 hours to 48 hours on source plants while only 39 per cent had fed for 3 hours or less on source plants. This resulted in a lower percentage of infection for the test plants in table 5 and it would be unfair to compare percentages from susceptible and from resistant source plants in this table with those in table 3. Most of the experiments included in the 156 tests involving the data in table 5 were set up under conditions which would not warrant the use of any data from them in connection with table 3.

difference was brought out more decidedly when strain 2 was used than when strain 1 was used.

The evidence shows conclusively that resistant sugar beet plants carry a much lower concentration of curly-top virus than similar plants of a susceptible variety when both are infected with the same virus strain.

Virus Concentration as Related to Virulence of the Virus Strain. Any comparison of strains 1 and 2 from susceptible source plants involves the consideration of the differences in severity of symptoms. It is believed that efforts to secure a fair comparison in the case of susceptible source plants were successful, and it is certain that dwarfing and distortion were not involved as factors when the resistant beet S.L. 68 was used as a source plant.

Tests on susceptible plants from susceptible source plants showed a greater virus concentration for plants infected with strain 1 than for those infected with strain 2 in a high percentage of the tests (Table 4). Of the

total susceptible test plants inoculated there was 11 per cent more infection among those inoculated from strain 1 source plants than among those inoculated from strain 2 source plants (Table 5).

Tests on resistant plants from susceptible source plants showed a still greater percentage of cases in which there was higher virus concentration for the plants carrying strain 1 (Table 4). Of the total resistant test plants inoculated there was 14 per cent more infection among those inoculated from strain 1 source plants than among those inoculated from strain 2 plants (Table 5).

Tests on susceptible plants from resistant source plants showed a higher virus concentration for plants carrying strain 1 than for those carrying strain 2 (Table 4). Of the total susceptible test plants inoculated there was 14 per cent more infection among those inoculated from strain 1 source plants than among those inoculated from strain 2 source plants (Table 5).

Tests on resistant plants from resistant source plants showed a higher virus concentration for the strain 1 source plant in every case (Table 4). Of the total resistant test plants inoculated there was 12 per cent more infection among those inoculated from plants carrying strain 1 than from those carrying strain 2 (Table 5).

When comparing strains 1 and 2 it again appears that differences in virus concentration were more clearly evident when the test plants were of the resistant beet variety than when the susceptible beet was used. This was true whether the source plant was susceptible or resistant, but the differences were more striking on either variety of test plant when the source plant was resistant. This might indicate an actually greater difference in concentration in the resistant source plants or that any existing difference is more strikingly brought out.

It is clearly evident that sugar-beet plants infected with strain 1 carry a higher virus concentration than those infected with strain 2.

Virus Concentration as Related to Time Elapsed after Infection. A sugar-beet plant which has been infected by curly top either dies in a few weeks or shows a more or less pronounced tendency to recover as evidenced by less severe symptoms on the new growth. This is true either in the field or in the greenhouse.

The source plants used in these tests were inoculated in the young two-leaf stage. Some plants which had been infected for 24 days were compared with others which had been infected for 5 months; plants infected for 5 weeks were compared with others infected for 5½ months; plants infected for 2 months were compared with others infected for 8 months; and some infected for 3 months were compared with others infected for 7 months. The plants infected for the longer periods in each group had definite signs of recovery. They were given additional nitrogenous fertilizer in the hope that this would tend to correct any effects that might be due to a deficiency in nutrients. The only tests in this entire series in which some source plants showed a higher concentration of virus strain 1 for the older plants (see

TABLE 6.—*Consolidated data from tests to determine the concentration of curly-top virus in source plants as related to time after infection*

Source and strain of virus and susceptibility of test plants	Source plant pairs[a]	Feeding periods	Total tests	Tests showing					
				No difference in virus concentration		Lower virus concentration in plants a few weeks after infection		Lower virus concentration in plant several months after infection	
	Number	*Number*	*Number*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Strain 1 virus									
Susceptible source plants									
Susceptible test plants	6	9	18	2	11	2	11	14	78
Resistant test plants	6	7	14	0	0	2	14	12	86
Resistant source plants									
Susceptible test plants	4	6	12	1	8	1	8	10	83
Resistant test plants	4	6	12	0	0	4	33	8	67
Strain 2 virus									
Susceptible source plants									
Susceptible test plants	4	4	10	0	0	0	0	10	100
Resistant test plants	4	4	10	0	0	0	0	10	100

[a] Each pair of source plants consisted of two sugar-beet plants of the same variety and infected with the same curly-top-virus strain, but one plant had been infected for more than 3 months and the other for a few weeks.

table 6) were among those in which the younger infections were 2 or 3 months old. This is undoubtedly explained by the fact that these plants were recovering.

Tests on susceptible plants from susceptible source plants carrying strain 1 showed a higher virus concentration for the younger infections in a very high percentage of the tests (Table 6). Of the total susceptible test plants inoculated there was 16 per cent more infection secured among those inoculated from plants which had been infected for a short time than among those which had been infected for a long time (Table 7).

TABLE 7.—*Summary of results of test-plant inoculations made during studies on age after infection as related to curly-top-virus concentration in sugar beet*

Source and strain of virus and relative age of plants after infection	Resistant test plants			Susceptible test plants		
	Inoculated	Diseased		Inoculated	Diseased	
	Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
Strain 1 virus						
Susceptible plants less than 3 months after infection	298	145	49	300	222	70
Susceptible plants more than 3 months after infection	297	67	23	307	165	54
Strain 1 virus						
Resistant plants less than 3 months after infection	251	88	35	251	181	72
Resistant plants more than 3 months after infection	254	59	23	257	134	52
Strain 2 virus						
Susceptible plants less than 3 months after infection	219	104	48	230	184	80
Susceptible plants more than 3 months after infection	228	38	17	230	119	52

Tests on resistant plants from susceptible source plants carrying strain 1 showed a higher virus concentration for the young infections in a still greater percentage of the trials (Table 6). Of the total resistant test plants inoculated there was 26 per cent more infection among those inoculated from plants which had been infected for a short time than among those which had been infected for a long time (Table 7).

Tests on susceptible plants from resistant source plants carrying strain 1 showed a higher virus concentration for the younger infections in a very high percentage of the tests (Table 6). Of the total susceptible test plants inoculated there was 20 per cent more infection secured among those inoculated from plants which had been infected for a short time than among those which had been infected for a long time (Table 7).

Tests on resistant plants from resistant source plants carrying strain 1 showed a higher virus concentration for the younger infections in a high percentage of the trials (Table 6). Of the total resistant test plants inoculated there was 12 per cent more infection secured among those inoculated from plants which had been infected for a short time than among those inoculated from plants which had been infected for a long time (Table 7).

Tests on susceptible plants from susceptible source plants carrying strain 2 showed a higher virus concentration for the younger infections in every case (Table 6). Of the total susceptible test plants inoculated there was 28 per cent more infection secured among those inoculated from plants which had been infected for a short time than among those inoculated from plants which had been infected for a long time (Table 7).

Tests on resistant plants from susceptible source plants carrying strain 2 showed a higher virus concentration for the younger infections in every trial (Table 6). Of the total resistant test plants inoculated there was 31 per cent more infection secured among those inoculated from plants which had been infected for a short time than among those inoculated from plants which had been infected for a long time.

It is clearly evident that, under the conditions of these experiments, the virus concentration was greater in plants a short time after infection than it was several months after infection. This was true for both strain 1 and strain 2 of the virus and regardless of whether the source plant was a resistant or a susceptible sugar beet.

DISCUSSION

It has already been shown (3) that age of plants at time of curly-top inoculation is an important factor in the amount of infection and the degree of injury which may occur. The data here presented indicate that the relative age of infection as related to virus concentration is another factor in favor of the older beets. Both the increased resistance and the decreased virus concentration are favorable factors in any beet fields in which there are few curly-top-infected beets and a leafhopper population that does not increase for some weeks. Such conditions may well be factors of importance in connection with beets planted for seed or in other plantings made in the fall when conditions become progressively less favorable for the leafhoppers.

The presence of the more virulent curly-top-virus strain 1 in any beet field is certain to result in more rapid spread of the disease than if the virus were strain 2, as evidenced by the greater concentration of the strain 1 in either the susceptible or the resistant sugar-beet source plants (Tables 4 and 5). Although other curly-top-virus strains producing relatively serious or slight injury to sugar beet have not been studied it is reasonable to expect that their comparative concentrations in infected sugar-beet plants would vary in a manner similar to those of strains 1 and 2.

The data in table 3 show that, under the conditions of these experiments, the percentage of infection secured from resistant source plants carrying strain 1 was only 23 when resistant test plants were used while the same virus from susceptible source plants gave 65 per cent infection among susceptible test plants. This would indicate that the rate of spread of strain 1 curly-top virus in a field of susceptible sugar beets might well be approximately three times as rapid as it would be in a field of the highly resistant beets, assuming that each field had the same amount of initial infection and

the same population and distribution of the vector. In the case of strain 2 virus there was only 12 per cent infection secured from resistant source plants when resistant test plants were used, while the same virus from susceptible source plants gave 72 per cent infection among susceptible test plants. This would suggest the possibility for six times as rapid a rate of spread of virus strain 2 among the plants in a field of susceptible beets as in a field of resistant beets.

Field conditions would rarely, if ever, be closely similar to those used in these experiments, but the general principles which brought about these results in the greenhouse would certainly be important factors in commercial beet fields. In other words, it is clear that the rate of spread is certain to be greatly reduced in fields of resistant beets and that the presence of susceptible beets adjacent to the resistant fields would greatly increase the rate of spread. Resistant sugar-beet varieties are well known for their ability to resist severe injury when infected and for the lower rate of infection that results when they are inoculated. The much lower virus concentration carried by such resistant beets is another important factor in their favor.

SUMMARY

The curly-top-virus concentration is much greater in infected susceptible sugar beets than in infected resistant sugar beets. This is true for both strain 1 which is highly virulent and strain 2 which is relatively low in virulence. The difference in virus concentration between resistant and susceptible plants is much greater in those infected with strain 2 than in those infected with strain 1.

The curly-top-virus concentration is much greater in sugar-beet plants infected with the highly virulent strain 1 than in those infected with the less virulent strain 2. This was true in both the susceptible sugar beet and the resistant sugar beet. The difference between strains 1 and 2 in virus concentration is much greater in resistant source plants than in susceptible source plants.

The curly-top-virus concentration is much greater in sugar-beet plants three weeks to twelve weeks after infection than in plants which have been infected for three to eight months. This is true when the source plants are infected with either virus strain 1 or with virus strain 2 and, in the case of strain 1, whether the source plant is of the resistant variety or the susceptible variety.

The χ^2 test shows high significance in the virus concentration differences found between infected plants of the resistant sugar-beet variety S.L. 68 and the susceptible variety S.L. 842; between plants infected by strain 1 virus and those infected by strain 2 virus; and between those which have been infected for 3 to 12 weeks and those which have been infected for 3 to 8 months.

The lower virus concentration in resistant sugar beets is a favorable

factor, reducing the rate of spread of curly-top infection among such varieties.

In all groups which were studied the resistant sugar-beet test plants gave more striking evidence of differences in virus concentration than the susceptbile test plants. This indicates the possibility of mass action as a factor related to infection (4).

DIVISION OF SUGAR PLANT INVESTIGATIONS,
U. S. DEPARTMENT OF AGRICULTURE.

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MASS ACTION AS A FACTOR IN CURLY-TOP-VIRUS INFECTION OF SUGAR BEET

N. J. GIDDINGS[1]

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INTRODUCTION

The term "mass action," as used in this article, is defined as the effect of the varying concentrations of the reacting masses on infection whether the reactions involved are reversible or not.

Carsner and Lackey (3) stated that the relation of mass action to curly-top infection has been shown: "(1) by varying the amount of inoculated virus by (a) inoculations with contrasting numbers of leafhoppers and (b) unequal periods of exposure; (2) by use of plants differing in susceptibility; (3) by studies on the incubation period of the virus in the insect; and (4) by comparing the minimal infective doses of the virus in its virulent and attenuated condition." They did not publish the data upon which these conclusions were based.

Severin (10) says "The relation of mass inoculation by groups of beet leafhoppers to curly-top transmission was demonstrated by varying the time of exposure of the insects on healthy beets." Study of his data suggests the possibility that mass action might be an infection factor but the evidence is not convincing.

There are many important variables which require consideration in the study of mass action as related to curly-top infection. Extreme variation among individual leafhoppers as to efficiency in transmitting the curly-top virus, *Ruga verrucosa* Carsner and Bennett, has been reported by Carsner and Stahl (4), Severin (10), Freitag (5), and Bennett and Wallace (2).

The use of different curly-top-virus strains or of mixtures of strains might readily account for extreme variation in results. It is certain that no consideration was given to strains in the earlier work, since their existence was not known at that time.

Comparisons between the amounts of infection produced by leafhoppers that had been fed on two different diseased plants might easily be misleading unless differences in virus strains or virus concentrations in those diseased plants are considered (9). Giddings (7) has reported that leafhoppers fed for a short time on diseased plants containing virus strain mixtures showed extreme irregularity in the strains that they transmitted and this is another source of variability.

While the conclusions reached in this paper are in general agreement with those of the earlier workers it is believed that the evidence presented herewith is such as to give those conclusions a far more substantial foundation.

[1] Senior Pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

The work reported in this paper was not undertaken for the purpose of learning the possible rôle of mass action as related to curly-top infection of sugar beets, and the data submitted are by-products of experiments dealing with virus concentration (9). A tabulation of results from a group of curly-top-virus-concentration experiments was found to furnish such strong evidence of mass action as a factor in infection that all such experiments were checked for similar evidence.

METHODS

The data were secured from sugar-beet plants of the highly resistant variety S.L. 68 and the very susceptible variety S.L. 842. The curly-top-virus strains involved were those previously described (6) as strain 1 and strain 2. The methods of feeding leafhoppers on source plants and of inoculating and handling the test plants are described in detail elsewhere (9). The periods of feeding on source plants were 30 minutes, 1 hour, and 2, 3, 6, 9, 15, and 24 hours. Each leafhopper then was allowed to feed for a week upon the cotyledon of one test plant.

If the experiments had been set up for the study of mass action the lengths of feeding period on infected (source) plants might have been somewhat different, the numbers of plants and leafhoppers involved would have been kept closely similar for each feeding period and some other variables would have been avoided. Since the data available appeared entirely adequate to prove the importance of mass action, it was deemed unwise to conduct further experiments merely in an effort to present a clear picture for each of the feeding periods mentioned. Therefore, the data from 30-minute feedings were combined with those from 1 hour, those from 2 hours with those from 3 hours, and those from 6 hours with those from 9 hours. In each case the longer period of feeding involved a much larger number of plants.

EXPERIMENTAL RESULTS

It was well known from earlier work (6) that the percentage of infection secured among inoculated beets of the resistant variety by either virus strain 1 or strain 2 was normally less than the percentage of infection among plants of susceptible varieties. This is further indicated by the results given in table 1 for controls; that is, inoculations with leafhoppers reared on source plants. If such a difference in amount of infection secured under controlled conditions were due to some factor other than mass action then the relative percentages of plants infected should remain proportional when the amount of inoculum carried by the leafhopper is greatly reduced. It is evident from the data in table 1 that such is not the case. The ratio of infections was 1:1.1 among resistant and among susceptible plants when using single leafhoppers that had been reared on infected plants, whereas the ratio was 1:2.9 for those leafhoppers that had been fed for one hour or less on an infected plant. The data for each of the successively shorter feeding periods show a striking consistency in the change of ratio.

The relation between length of feeding time on source plant and percentage of infection among inoculated plants (Table 1, columns 4 and 7) is also believed to be significant. The low percentage of infection secured from the short feeding periods might be partially explained by the possibility that some of the leafhoppers had failed to feed on the source plant, or had obtained only water from it during the 1-hour or 3-hour feeding periods but this could hardly be an important factor in the longer feeding periods. Bennett (1) has shown that very few leafhoppers survive more than two or three days at room temperatures if feeding is limited to parenchyma tissues or tap water, so it is reasonably certain that an appreciable quantity of the curly-top virus

TABLE 1.—*Evidence of mass action as indicated by differences in extent of infection of resistant and susceptible test plants in relation to period of feeding of vector on curly-top virus source plants*[a]

Feeding period on virus source plant	Resistant test plants			Susceptible test plants			Ratio of infected resistant to infected susceptible
	Inoculated	Infected	Infected	Inoculated	Infected	Infected	
	Number	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	
1 hour[b]	1703	109	6.4	1714	317	18.4	1: 2.9
3 hours[c]	1994	431	21.6	2036	1063	52.2	1: 2.4
9 hours[d]	624	212	34.0	631	444	70.4	1: 2.1
15 hours	227	89	39.2	232	159	68.5	1: 1.7
24 hours	231	121	52.0	223	168	75.3	1: 1.4
Controls (reared on source plant)	1538	1228	79.8	1566	1433	91.5	1: 1.1

[a] The data in this table were submitted to the χ^2 test which indicated highly significant differences between the infection percentages.
[b] Combined data for 30 minutes and 1 hour.
[c] Combined data for 2 hours and 3 hours.
[d] Combined data for 6 hours and 9 hours.

was picked up by every leafhopper which fed 15 or 24 hours upon the source plant. During one week of feeding upon the test plant it seems highly probable that such a leafhopper vector would inject at least a few virus particles into this plant and that the plant would become infected if a small number of virus particles could induce infection. Both susceptible and resistant test plants show successively large increases in the percentage of plants infected by leafhoppers fed for these longer periods and for the controls (Table 1), indicating that mass action must be a factor.

DISCUSSION AND SUMMARY

The data presented in this paper show very strong evidence that mass action is an important factor in the curly-top infection of either the resistant sugar-beet variety S.L. 68 or the susceptible variety S.L. 842. The reactions involved are initiated by the introduction of the curly-top virus into the plant. If the active mass of the virus introduced into the plant is sufficient to induce predominant reactions the final result is multiplication of the virus

and consequent infection of the plant. A smaller active mass of virus initiates the same reactions as the larger active mass but, because of the inadequate amount, the degree of the reaction is so limited that the final result does not permit multiplication of the virus. According to this hypothesis, the amount of active mass of virus required to bring about infection would vary with different beet plants and especially with different beet varieties because of the differences in the active mass of the specific reacting substances in the plant. It would appear probable that the reactions involved in the case of curly-tip virus are similar to those which occur in many bacterial and fungus infections in which mass action is a factor.

If mass action is a factor in the case of beet varieties S.L. 68 and S.L. 842, it is undoubtedly a factor for other beet varieties and for other plant species.

Evidence from field experiments on age of plants as a factor in resistance to curly top (8) gives support to the mass action concept. It would seem that, as the plants become older, there is an increase in them of the active mass with which the virus active mass must react before virus multiplication may take place.

The evidence that mass action is an important factor in infection should give added stimulus to the effort to reduce the amount of curly-top virus available in the vicinity of commercial fields of sugar beets or of other crops which suffer from the curly-top disease. The growing of curly-top-resistant beets and the elimination of weed plants which serve as virus reservoirs can be of great value in decreasing the amount of virus available to the insect vector. This would result in a smaller relative mass of inoculum and a consequent less amount of curly-top infection of beets and other crops in areas where this disease is prevalent.

DIVISION OF SUGAR PLANT INVESTIGATIONS,
U. S. DEPARTMENT OF AGRICULTURE,
RIVERSIDE, CALIFORNIA.

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STRIPE SMUT (USTILAGO STRIAEFORMIS), IN RELATION TO BLUEGRASS IMPROVEMENT[1]

J. G. Leach, C. V. Lowther, and Mary A. Ryan

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In 1940 the West Virginia Agricultural Experiment Station, in cooperation with the U. S. Regional Pasture Research Laboratory, undertook a program of bluegrass (*Poa pratensis* L.) improvement as part of a broader program of pasture improvement. Among the objectives of the project were studies of variation within the species, the selection of types best adapted to specific systems of management, and the breeding of strains with desirable characters, including resistance to disease. Preliminary studies (10) already reported have shown that there was much variation among individual-plant selections of bluegrass when they were grown in a nursery for observation. Mildew, rust, smut, and several fungus leaf spots were prevalent in the nursery, and differences in resistance of individual plants to the various diseases were obvious. In the nursery where the grass was not kept clipped some of these diseases appeared to be more prevalent than they are in most pastures. Further observation indicated that this was true for mildew, rust, and most leaf spots, but not for smut. Comparisons made of the prevalence of smut in the nursery and in pastures showed a close correlation between its presence in the nursery collections and its presence in the pastures from which the nursery collections were made.

The greater prevalence of rust, mildew, and leaf spots in the nursery as compared to that in pastures can be explained by the greater opportunity for building up inoculum on the unclipped grass of the nursery than on the grazed grass of the pastures. Because of the systemic nature of smut infection, its development and spread are less dependent on unrestricted growth of the plant than are rust, mildew, and the leaf spots. On the other hand there is some reason to believe that development of smut and its spread into new tillers may be stimulated by clipping or grazing. These facts all lead to the conclusion that smut is potentially the most destructive disease of bluegrass in pastures.

Pasture surveys made in West Virginia and in Pennsylvania have borne out this conclusion (10). No pastures were found to be entirely free from smut, and by actual count of representative quadrats several pastures were found in which more than 25 per cent of all bluegrass plants were affected. Large areas with more than 50 per cent infection are not uncommon. In dry weather many of these plants die, resulting in poor pastures that otherwise would be quite productive. It is obvious that smut resistance must be given prime consideration in any program of bluegrass improvement. It is probable that in the mixed population found in a pasture many individual plants

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are resistant, while others are very susceptible. Should a small number of apomictic lines be selected without regard to smut resistance and be introduced as improved strains, they might easily prove worthless in the presence of smut infection.

Because of the prevalence and destructive nature of smut in present-day pastures and its still greater potential importance, a study of the disease was undertaken with particular reference to the problem of testing strains of bluegrass for smut resistance. It is a recognized principle that in breeding for resistance to a disease a technique of producing infection approaching 100 per cent on susceptible varieties is necessary for satisfactory elimination of susceptible strains. Since bluegrass smut had not been extensively investigated from this viewpoint no satisfactory technique was available, although some inoculation experiments had been reported.

LITERATURE REVIEW

Ustilago striaeformis (West.) Niessl is recognized as a composite species affecting many grasses. There is abundant evidence (3, 4, 5) that it is physiologically specialized on the various grass hosts, although the exact host range of each physiologic form has never been determined. Davis (4) recognized four physiologic forms (or races) as follows: forma *Phlei* on *Phleum pratense* L.; forma *Agrostidis* on *Agrostis palustris* Huds.; forma *Poae-pratensis* on *Poa pratensis* L., and forma *Poae-annuae* on *Poa annua* L. The stripe smut on orchard grass was given specific rank and named *Ustilago clintoniana*. Fischer (5) studied a race of the smut affecting species of *Agropyron, Elymus, Hordeum,* and *Sitanion* and designated it as forma *Hordei.*

While these different physiologic races of the smut are morphologically and symptomatically similar they appear to differ not only in their parasitic specificity, but also in methods of chlamydospore germination, methods of infection, and in cultural characteristics. Davis (2, 4) working with spores from smutted timothy, red top, bluegrass, and orchard grass concluded that they would not germinate until they had passed through a resting period of about 240 days. He stated that the type of germination was the same for spores from all four host plants. "The promycelia were at first unicellular and multinucleate, but under certain conditions became multicellular with four to five lateral sporidia; however, occasionally only one lateral sporidium formed on the sides. The granular protoplasm assembled in the tips of unicellular promycelia, which sometimes formed lateral sporidia. Secondary spores or buds were usually formed from conidia, primary sporidia, tips of promycelia and other buds. . . . Lateral sporidia sometimes fused and formed conidia which developed buds and mycelial threads." Davis was unable to culture the fungus in decoctions, agars, and other media.

Fischer (5), on the other hand, found no after-ripening period necessary for germination of chlamydospores of forma *Hordei.* The process of germination also differed from that described by Davis. Two or three thick germ

tubes emerged from each spore. These developed into branched septate promycelia from which typical elliptical sporidia were budded in profusion. The sporidia grew and developed into large sporidial colonies on nutrient agar. This was the first record of the cultivation of *Ustilago striaeformis* on artificial media. The sporidia were found to be unisexual and fusions occurred when sporidia of opposite sex were mixed together on nutrient agar. Long, vigorous aerial infection hyphae arose from each fused pair of sporidia.

Kreitlow (6), working with forma *Agrostidis,* found that fresh chlamydospores usually did not germinate, although one collection of spores germinated without a rest period. Later (7) he was able to break the rest period of spores from *Poa pratensis* by subjecting them to a temperature of 35° C. in a moist chamber for 25 or 30 days. Spores of forma *Agrostidis,* according to Kreitlow, germinated by forming a single branched or unbranched promycelium 20 to 50 microns in length. No sporidia were observed, although lateral branches resembling sporidia were observed on some rapidly growing promycelia. The method of germination of the chlamydospores from bluegrass was not described.

Kreitlow (6) found that germinating chlamydospores of forma *Agrostidis,* when transferred to agar, grew readily, but formed strictly mycelial colonies in contrast to the sporidial growth obtained by Fischer (5) with forma *Hordei.*

Davis (2, 4), after extensive inoculation experiments with the smut on several different hosts, concluded that infection occurred in the seedling stage, penetration taking place through the coleoptile. Efforts to inoculate other tissues of growing plants were unsuccessful. Floral infection was not obtained and there was no evidence that the smut was seed borne. Fischer (5), on the other hand, found that seed taken from smut-infected plants of slender wheat grass produced only smutted plants, indicating that, on this host at least, the fungus is seed-borne.

Such wide variation in the behavior of the smut, as reported on various hosts by different investigators, would make it unwise to generalize too much about the life history of *Ustilago striaeformis.* Since the smut has been studied less extensively on bluegrass than on almost any other host plant, a thorough investigation of its life history on this host seemed necessary as a basis for working out any practical method of artificial inoculation.

EXPERIMENTAL RESULTS

Chlamydospore Germination and Culture on Artificial Media

The work reported here deals primarily with a study of the fungus in artificial culture and with inoculation experiments in the greenhouse. Abstracts of certain aspects of the work have been published (8, 9).

Life-history studies were started with attempts to germinate the chlamydospores. Spores from infected plants growing in the nursery were collected in late summer and attempts were made to germinate them in distilled

water and various nutrient solutions, as well as in and on the surface of plain and nutrient agar. Very few spores (never as much as 1 per cent) germi-

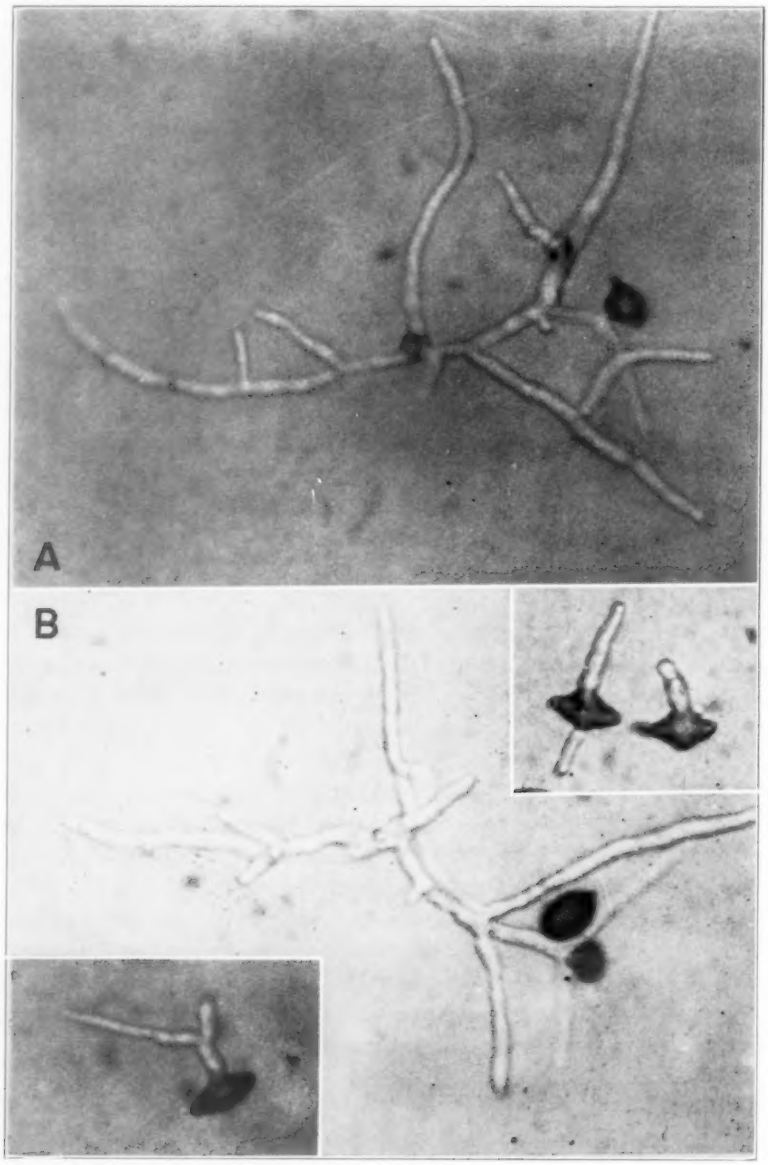

FIG. 1. Chlamydospores of *U. striaeformis* germinating in 2 per cent malt extract. The promycelium is indeterminate, the germ tube is branched, and sporidia are lacking. ×640. A, spores from bluegrass; B, spores produced in artificial culture.

nated under any of the conditions provided and there were no significant differences between the amounts of germination on the various media used. On the assumption that failure of the spores to germinate was caused by an

acquired dormancy, attempts were made to germinate young spores taken from unruptured pustules. This was done on the theory that dormancy might be induced by some change in the permeability of the spore wall after exposure to the air. Fresh leaves showing early stages of infection were taken from greenhouse plants and surface sterilized in a mercuric chloride solution, (1–1000), and then washed in sterile distilled water. Sections of the leaf were cut aseptically through a young unruptured smut pustule. These leaf sections were placed in the bottom of a sterile Petri dish in a drop of sterile distilled water and cut into numerous small segments with a sterile scalpel. A tube of melted agar was then poured over the segments and the plate incubated at room temperature. Microscopic examination showed that numerous chlamydospores had been released from the pustules and were distributed throughout the agar. These spores did not germinate much better than those used in previous studies, but the few that did germinate grew in the agar and could be picked up aseptically and transferred to tubes of agar for further growth.

The chlamydospores germinated by forming one, or occasionally two or three, simple or branched germ tubes with no sporidia. In distilled water the germ tubes are usually unbranched or sparsely branched, but in agar or in nutrient solutions the tubes branched profusely (Fig. 1, A) and grew into characteristic colonies. Typical promycelia and sporidia have not been observed.

Even though only a few chlamydospores germinated, numerous small fungus colonies appeared in the agar near and adjacent to the smut pustules (Fig. 2, C). Since these resembled the colonies arising from germinating chlamydospores they were objects of interest. Further study showed that these colonies arose from fragments of smut mycelium from the leaf tissue adjacent to smut sori. Numerous colonies were picked out of the agar and subcultured on agar where they grew slowly into small white bead-like colonies when crowded (Fig. 3, E) or into larger colonies with a characteristic convolute surface when isolated (Fig. 3, A). When the colonies were examined microscopically most of them were found to be composed of a mycelium that broke up readily into short, sporidia-like fragments. These sporidia-like bodies, unlike most smut sporidia, do not multiply by budding; but each fragment, by apical growth, may form more fragmenting mycelium (Fig. 2, D, E). On subculturing these fragments an occasional, typical, mycelial colony appeared. The mycelial colonies formed a tough, velvety mat and the hyphae showed no signs of fragmentation. The two types of colony could usually be detected in very early stages of growth by microscopic examination. The young fragmenting colonies tended to be compact and the individual hyphae were characteristically curved and twisted, while the mycelial colonies were more spreading with straight hyphal branches (Fig. 2, A, B). Older colonies of the two types are shown in figure 3, A–D.

On subculturing the two types of colonies there was a tendency of the fragmenting colonies to dissociate and produce the typical mycelial colonies,

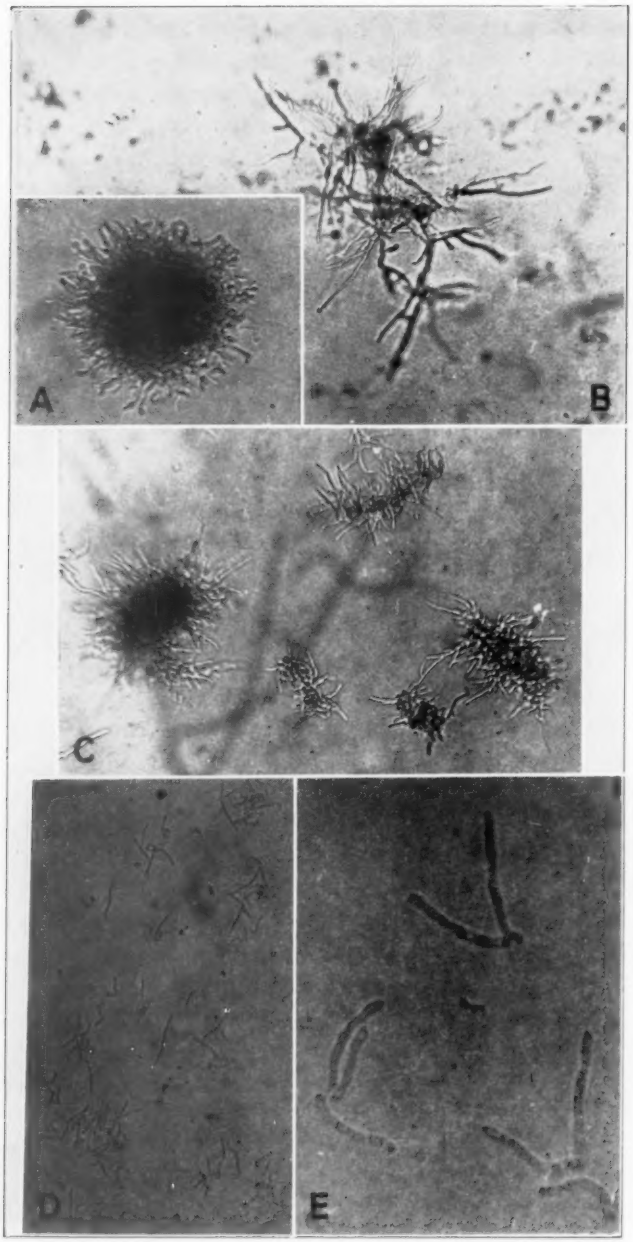

FIG. 2. Young colonies of *U. striaeformis:* A, fragmenting type; B, mycelial type; C, fragmenting type originating from mycelial fragments freed from immature pustules of smut plated on potato-dextrose agar. D and E, mycelial fragments of which a fragmenting colony is composed. A through D, ×125. E, ×560.

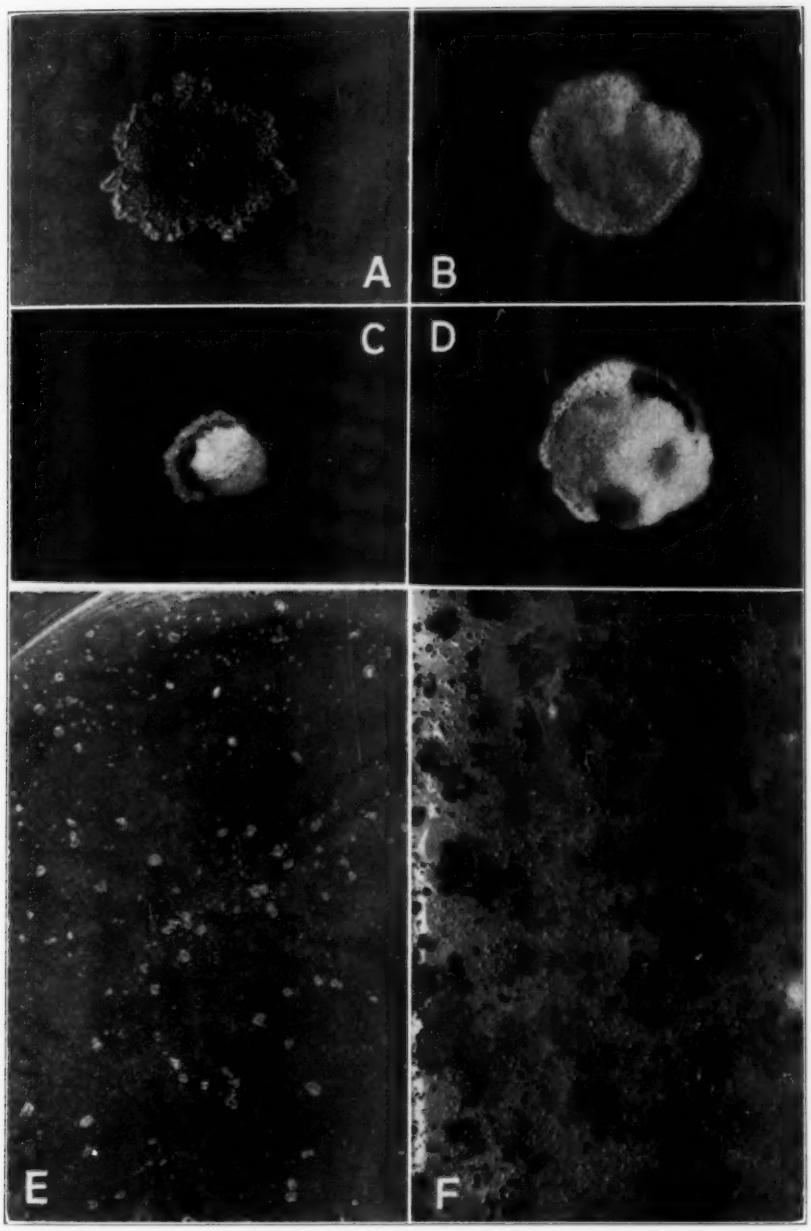

FIG. 3. Macroscopic view of colonies of *U. striaeformis*. A, fragmenting type in which the mycelium breaks up into short sporidia-like fragments. B, mycelial type in which there is no fragmentation of mycelium. C, a fragmenting colony that has produced a mycelial sector. D, a mycelial colony beginning to form chlamydospores in localized areas. A through D, giant colonies, natural size. E, small white bead-like fragmenting colonies arising from mycelial fragments isolated from immature smut pustules. Natural size. F, black masses of chlamydospores formed on the surface of an agar plate seeded with mycelial fragments from a fragmenting colony. The culture in early stages formed white bead-like fragmenting colonies as in E. In spore formation practically all of the mycelial fragments are transformed into chlamydospores. Slightly enlarged.

either as sectors (Fig. 3, C) or as individual colonies from separation plates. Both types of colony grew very slowly when first isolated, but after several subcultures there was an appreciable increase in the rate of growth as if the fungus had adapted itself to growing in artificial media.

After several subcultures on potato-dextrose agar some of the cultures of the fragmenting type changed from the usual glistening white to intense black (Fig. 3, F). On microscopic examination it was found that chlamydospores were being produced in abundance. The spores were similar to those formed on the host plant, but some of them were not typical in all respects. Chlamydospores produced in culture tended to be slightly larger than those produced on the host plant and the echinulations, although present, were not so clearly defined. Also there was usually a number of abnormally shaped spores; many of them tended to be more oblong than normal and many were lemon-shaped with characteristic pointed ends (Fig. 4).

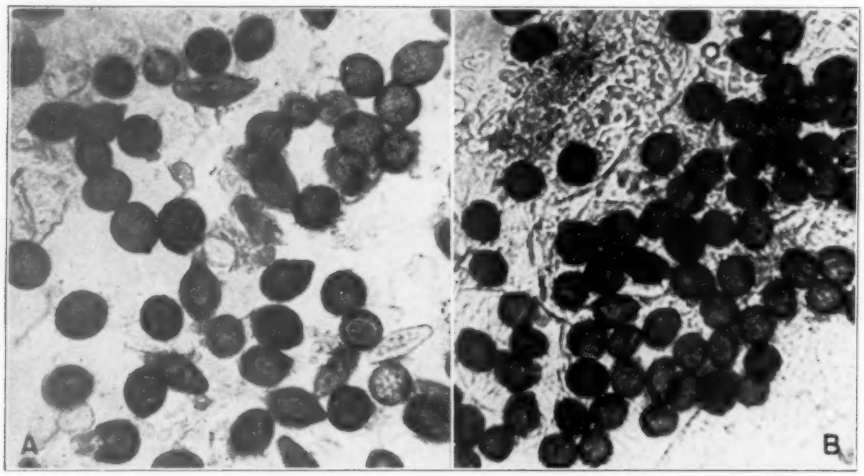

FIG. 4. Chlamydospores of *U. striaeformis:* A, produced on potato-dextrose agar; B, produced on bluegrass. ×500.

Sporulation at first occurred only in fragmenting colonies, but later certain typical mycelial colonies began to form chlamydospores (Fig. 3, D). Also certain isolates tended to form spores, while others seemed unable to do so. However, many of the apparent "nonsporulating" cultures suddenly began to sporulate after numerous subcultures had failed to form spores.

At first it appeared that the chlamydospores formed in culture would not germinate, but while the work was in progress Kreitlow (7) reported his success in inducing germination by subjecting the chlamydospores to a temperature of 35° C. in a moist chamber. When this method was tried on chlamydospores produced in culture they germinated as well as those produced on the host plant, and no difference in the methods of germination was observed (Fig. 1, B). Later some lots of spores from culture germinated fairly well without treatment, ranging from 5 to 50 per cent in different trials.

The significance of the various types of growth on artificial media has not been fully determined. Cytological studies are under way and the results will be published in another paper.

Inoculation Experiments

While the fungus was being studied in culture, inoculation experiments were being made in the greenhouse. Inasmuch as the experience of other workers had indicated seedling infection to be the general rule for this smut, seed inoculation was the first method tried. Fresh chlamydospores obtained from the field in late summer were used. At this time no means of inducing a high percentage of spore germination was known and the spores used germinated less than 1 per cent in distilled water. Several methods of seed inoculation were tried. One lot of seed was dusted with dry spores immediately before planting, another lot was soaked for two hours in a suspension of spores before planting. A third lot was soaked for two hours in a spore suspension during which it was subjected periodically to a moderate vacuum.

TABLE 1.—*Results of seed inoculation of bluegrass with fresh chlamydospores of Ustilago striaeformis, obtained from infected grass*

Method of inoculation	Percentage of infection
Dry spores dusted on seeds	2
Seeds soaked 2 hr. in spore suspension	0
Seeds soaked 2 hr. in spore suspension with vacuum treatment	3
Dry seed, no inoculation	0

Several hundred seeds of each lot were planted in rows in greenhouse flats. No infection was observed after six weeks' growth, at which time 100 plants of each treatment were transplanted into separate flats for further observation. The plants remained in these flats for about 5 months during which time a few of them became infected. The plants were then transplanted into the field for further observation, but the percentage of infection did not increase. The results of one representative set of seed inoculations are given in table 1. This method of inoculation was not very effective and, unless it can be greatly improved, it would be of no practical value in the selection of resistant types. The low percentage of infection obtained was assumed to be due to the failure of a sufficient number of chlamydospores to germinate at the proper time for infection.

Some time later, after the fungus had been isolated in pure culture on agar, a similar experiment was made comparing fresh chlamydospores from grass with inoculum grown on agar. The latter inoculum was suspended in water in a small flask with glass beads. By shaking the flask the clumps of spores or mycelium were separated into a fairly uniform suspension. A similar suspension of spores was prepared from smutted bluegrass leaves. The two suspensions were then transferred to the grass seeds in small vials in duplicate. One set of seeds was allowed to soak for two hours and then

the suspension was poured off and the seeds planted. The other set was placed in a vacuum for two hours during which time the vacuum was released momentarily four times. Two lots of seeds were given the same treatment without inoculum and used as checks. The eight lots of seeds were then planted in flats of greenhouse soil. One month after planting, 100 plants from each treatment were transplanted into flats where they were kept under observation for six months. The results were almost entirely negative, only one of the 600 inoculated plants becoming infected, this being one from the lot of seeds inoculated with mycelium fragments from an agar culture and the seed soaked 2 hours with no vacuum.

With the poor results obtained by seed inoculation it was necessary to try other methods. Inasmuch as chlamydospores formed in nature probably lie in the soil some time before germination it was decided to try soil inoculation.

On July 2 six 10-inch pots of soil were prepared. Two pots were thoroughly drenched with a suspension of fresh smut spores. Two pots were drenched with a suspension of spores and mycelial fragments from a spore-forming culture of smut grown on agar. The remaining two pots were not inoculated and were retained as checks. One pot of each pair was planted immediately with bluegrass seed. All pots were imbedded in the soil in an outdoor cold frame where they remained until October 4 when they were returned to the greenhouse. At this time the second pot of each pair was planted with bluegrass.

On October 8 the plants of the early planting were examined for infection and all plants showing no infection were then transplanted to flats of clean soil for further observation. They were examined at frequent intervals until the following June when the experiment was discontinued. Whenever an infected plant was found it was recorded and removed from the pot. Records were kept on the second planting until the following September. The results of this experiment are given in table 2.

It is evident from the results of these experiments that a much higher percentage of infection was obtained by soil inoculation than by seed inoculation. The most infection was obtained when chlamydospores from infected plants were used as inoculum. Inoculum grown on agar was much less effective, although some infection was obtained. It should be noted that infection was very slow in developing and that it required over 300 days for infection to show on some plants. It is not known whether this long time is due to a long incubation period within the plant, or whether the fungus persisted in the soil as mycelium or viable chlamydospores and infected the new tillers formed as the plants grew. Since the plants were transplanted into clean soil after 98 days it might be assumed that they were infected before transplanting, but as some soil was transferred with the plants some inoculum also could have been transferred.

In an effort to determine whether the fungus would persist in the soil and be capable of causing infection for an extended period, pot No. 1 was replanted without further inoculation on October 8 when the original plants

were transplanted. The new plants were allowed to grow until March 10 of the following year when they were removed and those showing no infection were transplanted into flats for further observation. On March 14 the pot was reseeded for the third time. The plants of the third crop were allowed to grow until July 22 when they were removed.

The infection occurring on these plants seeded into the previously inoculated soil was as follows: the first replant, or the second crop of plants, seeded 98 days after inoculation of the soil, produced 17 infected plants out of a total of 71 plants, or 23.9 per cent after 229 days. The second replant seeded

TABLE 2.—*The results of soil inoculation with two kinds of inoculum of U. striaeformis on bluegrass*

Pot No.	Inoculum and inoculation procedure	Percentage of infection	Days after planting
1	Fresh spores from infected plants; seeded immediately	25.30	98
		54.80	217
		69.90	250
		77.40	272
		87.00	327
2	Fresh spores from infected plant; seeded 3 months after soil inoculation	5.70	123
		27.00	155
		33.30	169
		52.00	208
		60.00	234
		62.80	246
3	Spores and mycelium from agar culture; seeded immediately	5 or 6 plants out of nearly 200 plants—exact percentage not determined	98
		No increase	280
4	Spores and mycelium from agar culture; seeded 3 months after soil inoculation	3.00	126
		9.10	172
		9.10	290
5	Check, no inoculum; planted immediately	0.00	240
6	Check, no inoculum; planted 3 months after soil inoculation	0.65	132
		1 plant out of 152	
		0.65	280

256 days after inoculation of the soil yielded 7 smutted plants out of 355 plants, or approximately 2 per cent after 100 days.

It is evident from these results that the inoculum does persist in some form in the soil under greenhouse conditions for as long as 256 days, although it decreases materially during this period. It is not possible to determine from this experiment whether the extremely long time required for the appearance of smut on some of the plants was due to the prolonged incubation period or whether infection occurred on older plants from soil-borne inoculum. Further experiments designed to answer this question are under way.

Working with *Ustilago striaeformis* on timothy, Davis (2, 4) concluded that seedlings were most susceptible when coleoptiles were 1 to 10 millimeters long. No infection was obtained by Davis after the coleoptiles were more than 16 millimeters long. The smut of bluegrass apparently is not

limited to such a short period of infection. Evidence that infection takes place on older seedlings of bluegrass was obtained as a by-product of an experiment designed to compare the effectiveness of different kinds of inoculum.

Seven glass culture dishes of moist sandy soil were steam-sterilized. On February 14, 1944, one dish was inoculated with a suspension of chlamydospores from bluegrass. A second dish was inoculated with mycelial fragments from a nonsporeforming culture grown on agar. A third dish was inoculated with a suspension of chlamydospores from an agar culture. One week later a second set of dishes was inoculated in the same way and all six dishes, together with an uninoculated check, were planted with bluegrass seed. Three weeks after planting 100 seedlings from each dish were transplanted to flats of soil where they were allowed to grow for six months, after which the experiment was discontinued. It is not necessary to tabulate the results of this experiment for only two of the 700 transplanted plants became infected. These were from the dish inoculated with spores from bluegrass one week before seeding. This experiment was planned on the assumption that, like the smut on timothy in the experiments of Davis (2, 4), infection would occur on the very young coleoptiles. As a matter of curiosity one dish, namely the one inoculated with spores from grass at time of planting, was saved and the plants were kept growing in the inoculated soil. On May 31, a little more than 3 months after planting, the plants were examined and 15 out of 337 were found infected. The 322 remaining plants were transplanted to flats and kept under observation until January 12, 1945, during which time a total of 78 or 23 per cent became infected. These results would indicate that the plants in the experiment were removed from the inoculated soil too early and that most of the infection occurred on plants more than three weeks old.

With this evidence that infection is not confined to very young seedlings, it was decided to try several other methods of inoculation. As a preliminary experiment twenty bluegrass plants growing in clean soil were selected, each of which had one or more young developing tillers. These were washed and the roots and tillers were dipped momentarily in a suspension of chlamydospores from bluegrass, after which they were transplanted into 4-inch pots of clean soil and frequently observed, so that infected plants were observed as soon as symptoms appeared. The records of this inoculation experiment are as follows:

105	days after inoculation,	2	plants infected	= 10 per cent
109	do	3	do	= 15 per cent
121	do	4	do	= 20 per cent
161	do	5	do	= 25 per cent
191	do	8	do	= 40 per cent
195	do	11	do	= 55 per cent
227	do	12	do	= 60 per cent

These results would indicate that infection is not confined to very young seedlings and that parts of older plants, probably young tillers, may become infected readily.

If infection is not limited to young seedlings it seemed possible that inoculum injected into growing plants at or near the growing point might prove effective. Accordingly, 25 vigorously growing seedlings were inoculated by injecting a suspension of fresh chlamydospores through the sheaths at the base of the stem near the growing point. This operation was per-

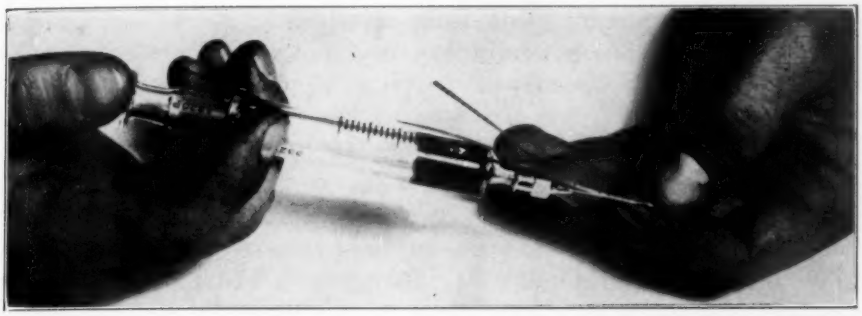

FIG. 5. The method used in inoculating bluegrass plants by injecting, with a hypodermic needle, a suspension of smut spores. An effort was made to place the spores as near as possible to the growing point without seriously injuring the plant.

formed by removing the plant from the soil and injecting the spore suspension with a hypodermic needle as shown in figure 5. The plants were then transplanted into flats or pots of soil and watered thoroughly. Infection was obtained as follows:

90	days after inoculation,	6	plants infected	= 24 per cent
123	do	9	do	= 36 per cent
145	do	10	do	= 40 per cent
242	do	12	do	= 48 per cent

This experiment showed that infection occurs when chlamydospores are injected with a hypodermic syringe into the stem of bluegrass plants. Although the avenue of infection is not known definitely, it is probable that the young leaf tissues at or near the growing point constitute the infection court.

When it was found that a relatively high percentage of infection could be obtained by injecting spores into the stems, similar inoculations were made using mycelium and chlamydospores from agar cultures, but very poor results were obtained. At the time these experiments were made all attempts to germinate the spores produced in agar cultures had failed.

In the meantime inoculation experiments have been devoted to improving the effectiveness of the hypodermic-needle method. This method has much promise in the development of a practical means of eliminating susceptible strains of bluegrass and identifying the resistant ones. If we were dependent upon seed inoculation, resistant strains could not be identified without some method of inoculation that would give practically 100 per cent infection on susceptible types. Otherwise, escapes could not be tested until seeds were produced and results of second generation tests would be complicated by genetic variations. With a method of inoculating relatively mature plants

there is no need of resorting to a second generation of plants from seeds. Bluegrass is easily propagated vegetatively and by inoculation with a hypodermic needle a single clone may be inoculated over and over again until its resistance or susceptibility is definitely determined.

As a trial demonstration of the practical use of this method of progressive elimination of susceptible plants 50 seedlings about 2 months old were inoculated with the hypodermic needle using a suspension of fresh spores from grass. Some of the plants were rather small and were injured during the inoculation and only 36 survived. Of these, 10 plants became infected as follows:

34	days after inoculation,	5	plants infected	= 13.9 per cent
37	do	8	do	= 22.2 per cent
39	do	9	do	= 25.0 per cent
77	do	10	do	= 27.7 per cent

By this time the plants had formed new tillers so 78 days after the original inoculation the 26 noninfected plants were divided into 3 to 5 separate plants and each plant was reinoculated with the hypodermic needle as before. Whenever one or more plants from a clone were found infected the entire clone was considered susceptible and discarded. Considering each clone as a plant the results of the second inoculation are given on a cumulative basis as follows:

21	days after second inoculation,	12	plants infected	= 33.3 per cent
24	do	14	do	= 39.0 per cent
26	do	15	do	= 41.1 per cent
29	do	16	do	= 44.4 per cent
37	do	20	do	= 55.5 per cent
58	do	20	do	= 55.5 per cent

The remaining 16 plants were again divided into clones of 6 to 10 individual plants and reinoculated with spores from grass that had been incubated for three weeks at 35° C. in a moist chamber. Tests showed about 5 per cent germination in water. Within 44 days after the third inoculation 12 more clones had been eliminated, leaving only 4 clones not infected. Three weeks later the remaining four clones were inoculated with fresh spores from grass. Three of these were eliminated within 43 days, leaving only one clone. This was divided into 18 plants and inoculated.

No infection developed in 70 days, so 18 plants were reinoculated and one plant developed smut after 59 days, making 100 per cent infection, thus eliminating them all as susceptible after a total interval of one year and 6 days.

This method of inoculation may seem too slow to be practical, but it is believed that, with further study, it can be speeded up considerably. Most of these inoculations were made with chlamydospores having low germination percentages (none more than 5 per cent). Some success in increasing the percentage of germination of spores used for inoculation has been obtained by using Kreitlow's (7) heat treatment, but results have been erratic. With further trials using spores with better germinating records the percentages of infection from a single set of inoculations should be increased.

In many of the experiments reported the grass grew very slowly and the slow growth may have contributed to the long incubation period. Although the incubation periods have been discouragingly long, they are, as a rule, shorter following hypodermic-needle inoculation than after any other method. The shortest incubation period recorded is 13 days following hypodermic-needle inoculation, while the shortest for seed or soil inoculation is 36 days.

When routine inoculation tests are made and when the only consideration is the elimination of susceptible clones, it should be possible to combine hypodermic-needle inoculation with soil inoculation and shorten the time interval considerably.

The foregoing experiments do not constitute all of the inoculation tests that have been made, but they are considered representative of the results obtained to date. Some inoculation tests have, for no explainable reason, failed to produce any infection, but, with further practice and experience, results become consistently more successful. Thus, in an experiment started on May 17, 1944, in which 148 plants were inoculated with the hypodermic needle using fresh spores from grass, 48.6 per cent of the plants became infected by January 12, 1945, as a result of the original inoculation. This is considered to be a fair average of success with the hypodermic-needle method alone.

Further experiments are under way with the aim of perfecting the method for practical use as well as to learn more about the methods of infection and life history of the fungus in nature.

DISCUSSION

Ustilago striaeformis on bluegrass differs in many respects from that on other grasses as described by Davis, Fischer, and others. The formation of typical chlamydospores on artificial media has not been reported previously for this organism, and is not common in other smut fungi, although Wang (11) has described a similar phenomenon for *Ustilago crameri* Keke. Because of this ability of the fungus to complete its entire life history on artificial media it lends itself well to cytological and genetic studies. Preliminary studies of this nature are under way and will be reported later.

The failure of the fungus to produce the usual type of sporidia and the occurrence of infection from soil-borne inoculum long after the seedling stage are in contrast to previously accepted concepts of the life history of the species. Its behavior in this respect is probably correlated with the growth habits of bluegrass. In nature the chlamydospores most likely germinate in the soil, forming a mycelium that is capable of infecting the young tillers, as well as young seedlings.

SUMMARY

1. *Ustilago striaeformis* from bluegrass is readily cultivated on artificial media. Chlamydospores are formed on agar in great abundance and, al-

though some of them are slightly abnormal in size and shape, they germinate normally, producing cultures indistinguishable from those arising from spores produced on bluegrass.

2. Chlamydospores germinate by forming one or more branched germtubes that grow into a mycelium on nutrient agar. Two types of colonies occur; one that breaks up into sporidia-like fragments, and one that is typically mycelial. Both types of growth may produce chlamydospores, but certain cultures of each growth type have not sporulated.

3. Conventional methods of seed inoculation resulted in low percentages of infection. Relatively good infection was obtained by soil inoculation and by injecting chlamydospores with a hypodermic needle into the stem near the growing point.

4. Infection from the soil is not confined to the young coleoptile, but may occur on older plants, probably through young tillers.

5. The practicability of inoculating vegetatively propagated clones of bluegrass has been demonstrated. It appears to be a promising means of eliminating susceptible clones and identifying resistant ones in a program of selecting or breeding smut-resistant strains of bluegrass.

WEST VIRGINIA AGRICULTURAL EXPERIMENT STATION,
MORGANTOWN, WEST VIRGINIA.

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TATTER LEAF OF SWEET CHERRY[1]

R. S. Willison and G. H. Berkeley

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In mid-June, 1940, advice was requested in connection with abnormalities in sweet cherry fruits, purported to be of the Black Tartarian variety, in an orchard near Niagara Falls, Ontario. On inspection, the fruit was found to be slightly smaller than normal and appeared to be ripening prematurely. The fruit surface was somewhat roughened with shallow pitting, in many cases centered around tiny black specks. The foliage showed considerable interveinal pallor. This condition, which has frequently been observed on cherry leaves in the spring and which is usually outgrown as the leaves mature, is regarded as being induced by growth factors at present obscure. In addition to this "growth mottle," however, the trees in question showed a faint blotching and chlorotic flecking suggestive of a mild virosis. Examination of fruits and leaves failed to disclose bacterial or fungal parasites that might account for the disease. The following season, symptoms on trees in this orchard ranged from traces of mottling alone to mottling accompanied by more or less laceration.

PRELIMINARY TESTS OF TRANSMISSIBILITY

In June, 1940, small pieces of bark from the smaller branches of one of the affected trees were patch-grafted on the trunk of a two-year Elberta peach tree and on two suckers of a thirteen-year-old Black Tartarian tree in the laboratory orchard. The inoculated cherry tree, in 1941, put out dwarfed shoots and small, ruffled leaves up and down the main branch bearing the inoculated shoots and on the adjacent side of the next main branch. The remainder of the tree was normal in appearance throughout the 1941 growing season. Affected leaves were marked interveinally with brown flecks, rings, lines, and streaks which soon dropped out, leaving a lacy network of tissue very often involving almost the whole leaf. Most of the lacerated leaves were retained throughout the summer, though the effects of the disease gradually became obscured by the development of new leaves, almost normal in size and either without markings or with faint patterns of the banded or outline "oak-leaf" type. A similar sequence of events has taken place in this tree in the growing seasons of 1942, 1943, and 1944. By 1942, the disease had spread through most of the inoculated tree, and in 1943 and 1944, there were indications that its effects were gradually decreasing in severity. The fruit symptoms noted on the original tree have not been observed on the inoculated one and may not have been caused by this disease.

Further evidence that the disease is graft-transmissible, and thus of virus origin, was obtained from the young inoculated Elberta tree, many leaves

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of which were marked with small rings and chlorotic and necrotic spots early in May, 1941. These symptoms, which were the only ones apparent in the first season after inoculation, did not occur in 1942 and subsequent years, when a variety of other symptoms appeared later in the season on a number of leaves scattered through the tree. These included faint but definite mosaic and oak-leaf patterns (Fig. 1, D), rings, and a curious premature aging of the upper leaf surface (Fig. 1, C) which appeared as if covered with a very thin, finely checked, dull, dirty yellow encrustation.

Since 1940, the disease, for which the name "tatter leaf" (11) seems to be appropriate, has been seen in several orchards in the Niagara Peninsula. Hildebrand and Palmiter (11) have observed cases of tatter leaf on sweet cherries in New York State. A similar condition, called "lace leaf" and considered to be distinct from cherry mottle leaf, has been reported from Washington State by Reeves (9) on the varieties Bing, Napoleon, and Deacon. Cochran (4, 5) also records lace leaf on sweet cherry following inoculation with a ring spot of peach.

THE DIFFERENTIAL HOST RANGE

After the preliminary demonstration that tatter leaf was graft-transmissible, the virus was transferred by budding, in 1941 and 1942, to the several varieties of the genus *Prunus* comprising the range of the differential hosts used in studying the virus diseases of stone fruits (12) namely: *Prunus domestica* var. Italian prune, German prune, Lombard, and Reine Claude; *P. salicina* var. Abundance; *P. persica* var. Rochester, Elberta, and seedlings; *P. avium* var. Napoleon (Royal Anne), Bing, Black Tartarian, and seedling; *P. cerasus* var. Montmorency; *P. cerasifera*, Myrobalan seedlings; and *P. mahaleb*, seedlings. Throughout the experiments, the Black Tartarian tree inoculated in 1940 was the source of the diseased buds used in making inoculations.

METHODS

The transfer of the virus to the respective differential hosts was effected in two ways: by the double-budding technique (12) and directly to nursery stock or to orchard trees. In double-budding, a bud from the diseased tree and one from the desired host variety were inserted on each of three stock plants, usually peach or Myrobalan seedlings, which were cut back to the upper grafted bud the following spring. This method has the advantage over direct inoculation of nursery stock of permitting the use of differential varieties of clonal origin, each indexed on peach, concurrently with inoculation, to ascertain its freedom from virus infection. The varietal clons, on which symptoms are described below and which were used in double-budding, were healthy unless otherwise specified. The nursery stock may or may not have been healthy, as it was not indexed because of lack of indexing stock, but it appeared to be normal throughout the summer of 1942 before inoculation.

VARIETAL REACTIONS

On Italian prune.—In the double-budding experiment on peach stock in 1941, transmission was indicated by symptoms on the peach foliage. The prune, however, showed only a faint mottle on a few leaves in the three subsequent seasons.

On the nursery stock, an indistinct mottle was observed on leaves near the point of inoculation in the first year, but not in the second.

On German prune.—Only the double-budding technique was used with German prune. The peach stock indicated a positive transfer of the tatter-leaf virus, but the prune remained symptomless for three seasons after inoculation.

On Lombard plum.—On nursery stock, the diseased cherry buds established union with the Lombard, but the only symptoms observed on the plum were some faint irregular pale markings (Fig. 2, G) or slight necrotic spotting and shotholing on a few leaves in the first season. The next year, very few faint patterns appeared.

On Reine Claude plum.—Definite symptoms occurred on the peach stock double-budded with Reine Claude plum and tatter-leaf cherry in 1941, but the only indication of infection on the plum was a very faint mottle, sometimes approaching the oak-leaf type of pattern, and appearing annually in early summer.

On Abundance plum.—After double-budding on Myrobalan stock in 1942, Abundance shoots grew vigorously, but, like the varieties of *P. domestica*, were not much affected by the virus. In the two years since inoculation, some mid-season leaves were perforated with a few scattered pinholes, a feature not observed on Abundance in other series. According to the results of indexing on peach, the Abundance clon, though apparently symptomless, was carrying a virus of the peach-yellows–little-peach group.

On Rochester peach.—Inoculation of this variety was made in 1942 on nursery stock only. At the beginning of the 1943 growing season, slight superficial bark necrosis was observed near the inserted buds, and some leaves were definitely marked with small confluent rings, either dark green with pale centers (Fig. 1, A) or pale green with dark centers (Fig. 1, B), and mostly in the proximal three-quarters of the leaf lamina. On some leaves, the rings were larger and more irregular, and on others, part of the surface was chlorotic and dotted with small circular to oblong green islands. These patterns disappeared later in the summer when, except for some laceration of older leaves, other symptoms were wanting. In 1944, none of the early sesaon symptoms appeared, but the foliage was somewhat dull and a few leaves were marked with the mid-season symptoms deseribed on Elberta. The first crop of fruit, in 1944, was only slightly reduced, if at all.

On Elberta peach and seedling.—The reaction of Elberta nursery stock to tatter leaf was similar to that of Rochester but somewhat less pronounced, except for the laceration of older leaves, which was preceded by the formation of red-rimmed, fawn-colored, necrotic areas.

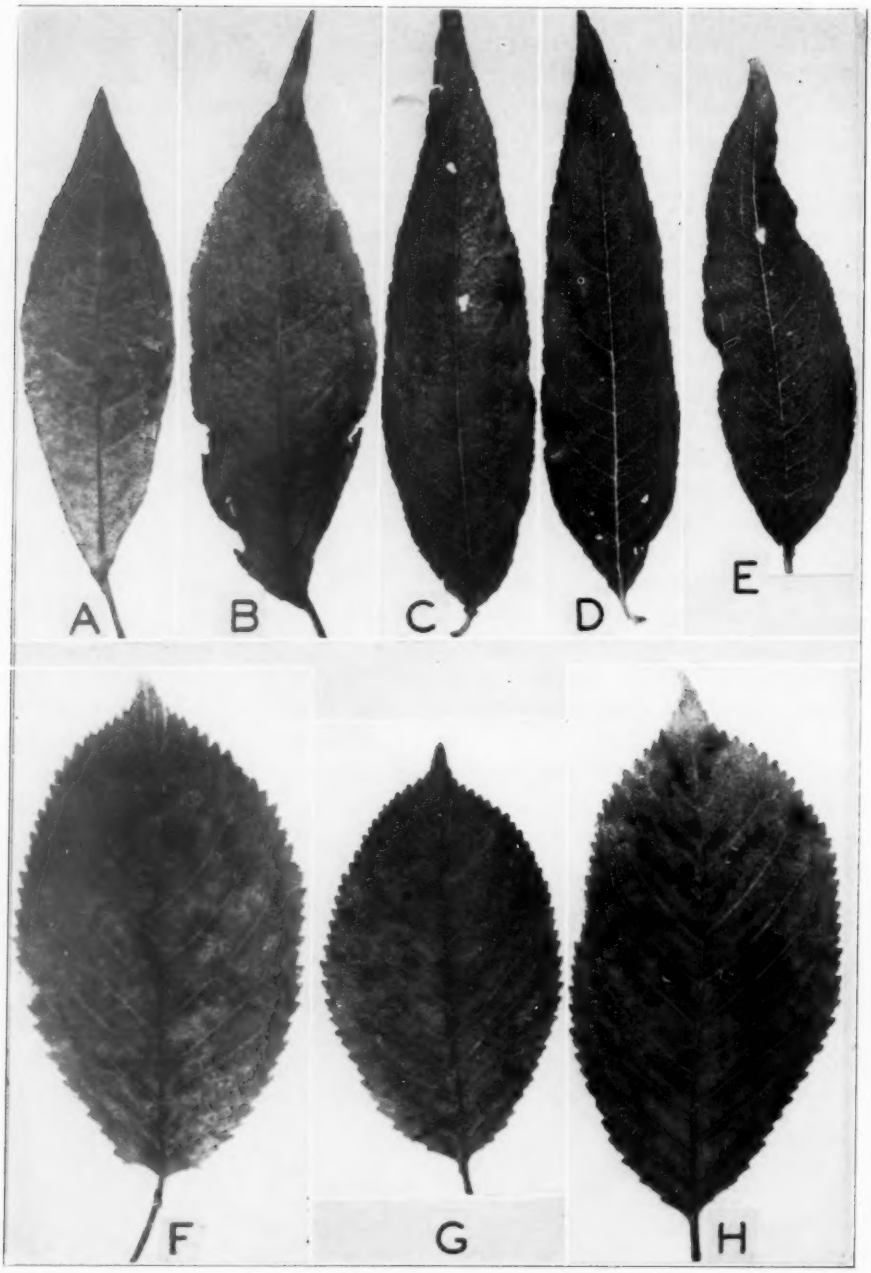

FIG. 1. Symptoms of tatter leaf on peach and Naploeon cherry. A–B. Acute symptoms on Rochester peach. C–E. Chronic symptoms on Elberta peach. F–H. Patterns and necrosis on Napoleon.

In the double-budding experiment of 1941, on the other hand, the characteristic early season symptoms were absent from the shoots both from the inserted Elberta bud and from the cut-back seedlings, but, as the leaves matured in the first season, their upper surfaces became somewhat lusterless and in some instances looked as though they had been lightly scoured with fine sand-paper near the margins. By the end of July, there had appeared the typical faint mosaic (Fig. 1, D), the surface aging (Fig. 1, C), and other patterns, which did not occur on the larger trees until the second summer. In addition, a number of leaves on most trees in this series were lightly sprinkled with a fine, persistent, red pin-spotting (Fig. 1, E). On the other hand, the fawn necroses and laceration of leaf tissue were wanting. The typical mid-season symptoms were also observed on these plants in the second and third years after inoculation. Some variations in the degree of symptom expression were noted on different peach seedlings.

On Black Tartarian cherry.—On nursery stock, some of the early leaves on a few spurs close to the inserted diseased buds were marked in the first season with fine brown lines (Fig. 2, A), roughly parallel to the main veins and outlining areas which later became necrotic (Fig. 2, B) and dropped out to give the typical lacy effect (Fig. 2, C, D). On Black Tartarian, the lacerations were interveinal and rarely if ever did they cross the main veins. Other leaves on the same spurs were faintly mottled with yellowish green. By the beginning of the second season, symptoms of both sorts were apparent on many leaves over about half the tree. In both years, leaves formed after the middle of June were symptomless. On the whole, tattering was much less severe on the nursery stock than on the orchard tree inoculated in 1940.

Late in August, 1941, a Black Tartarian tree of the same age as that used in the preliminary tests was inoculated by budding on shoots near the base of a main branch. Typical brown line markings and tattering were observed in the spring of 1942 but only on the inoculated shoot. The disease did not spread completely through the tree until 1944, when tattered leaves were relatively few, compared with those marked with a modified form of oak-leaf pattern. These symptoms appeared on leaves scattered here and there all over the tree.

On Napoleon cherry.—The only symptoms on Napoleon nursery stock in 1943 were observed early in the season near the point of inoculation. A few leaves were marked with small, pale green to chlorotic rings (Fig. 1, F), and some with larger, pale green rings or green-rimmed irregular pale areas (Fig. 1, G). Most of the patterns were faint and transient. A few necrotic spots, intervenial necrotic streaks (Fig. 1, H), and lacerations occurred on some leaves, but the tattering effect was less than on Black Tartarian. Some coarse, chlorotic lines and rings appeared in the second season over a larger area of the tree, but the necrotic markings and tattering were again inconspicuous.

On Bing cherry.—A small branch of a large, bearing tree was inoculated in August, 1941. The following spring, the first leaves to open on the inocu-

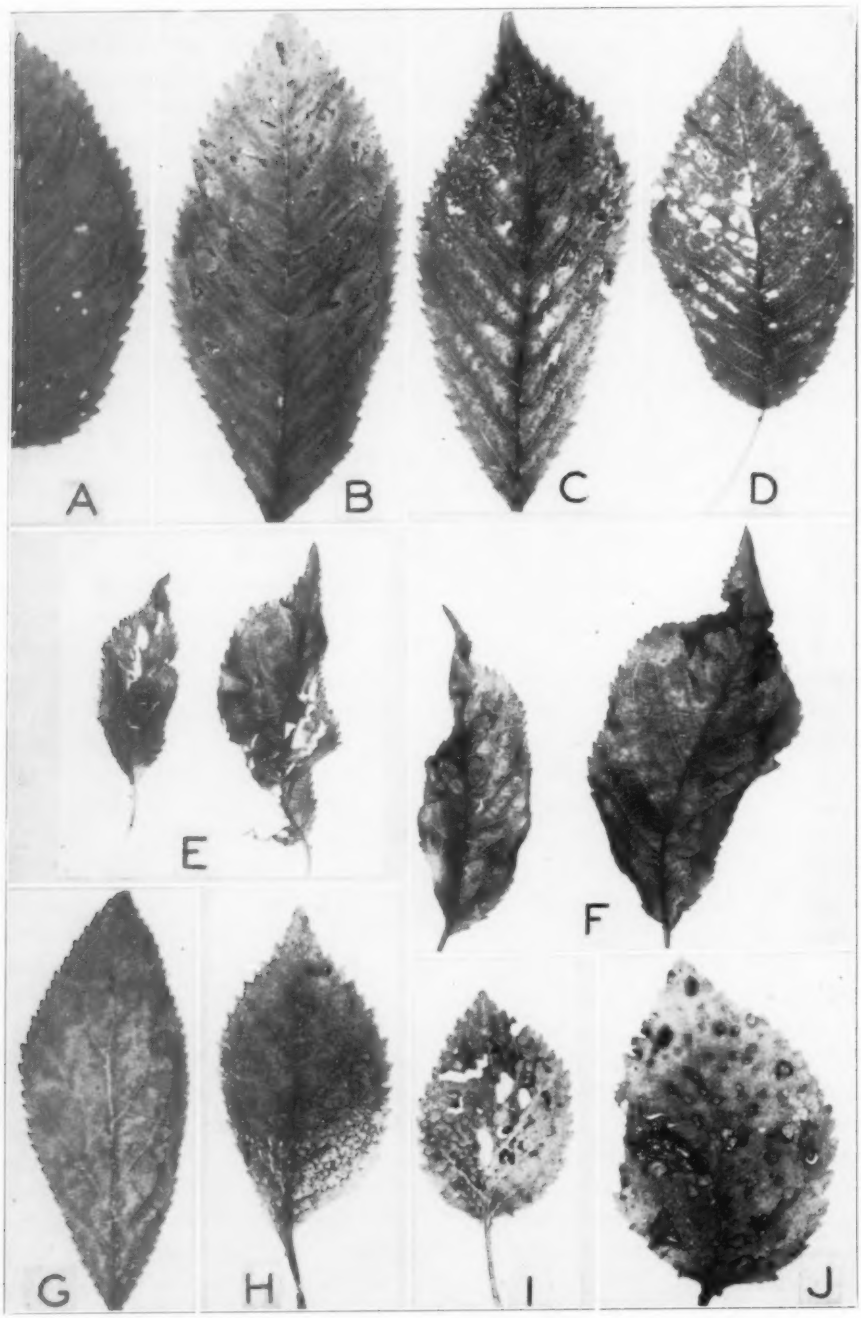

FIG. 2. Symptoms of tatter leaf on sweet cherry (Black Tartarian and Bing), on sour cherry (Montmorency), and on Plum (Lombard). A–D. Stages in the development of tattering on Black Tartarian. E–F. Necrosis, tattering, and ruffling in Bing. G. Mild mottle on Lombard. H–J. Ring and necrotic spotting on Montmorency.

lated shoot displayed some mottling and fine brownish etched lines quickly passing over into necroses (Fig. 2, F) and laceration (Fig. 2, E), which, instead of being interveinal as in Black Tartarian, involved larger areas of the leaf surface and often extended across main veins. Affected leaves tended to be ruffled and twisted (Fig. 2, E, F). No further symptoms appeared that season, but by the spring of 1943 the disease became evident on nearby branches, and by 1944 had spread throughout the tree. Symptoms in the second and third seasons varied from rings, mild patterns of the oak-leaf type, and mosaic mottling to necrosis, laceration, and distortion.

On a sweet cherry seedling.—A seedling of Elkhorn parentage was inoculated in the fall of 1941. Early in the next season, the seedling was cut back and the early leaves both of the seedling and of the inserted diseased Black Tartarian shoot were mildly but definitely mottled, but the characteristic lacerations failed to appear. In 1943 and 1944 a few leaves of both shoots were slightly tattered, but the predominating symptom was again the mild mottling, in either a mosaic or an oak-leaf pattern.

On Montmorency cherry.—The first symptoms to appear on the foliage of Montmorency nursery stock in the spring after inoculation were fine, translucent to dark brown, etched rings in all sizes up to about two millimeters in diameter (Fig. 2, H, I). The larger rings tended to be complete and regular, though frequently in tangential contact (Fig. 2, I). On the other hand, the smaller ones were often incomplete, confluent, and crowded to make a network tracery (Fig. 2, H). Numerous fawn-colored and usually isolated necrotic spots of various shapes and sizes, delimited by fine, red-brown lines (Fig. 2, J), were also present very early in the season, both on ring-spot leaves and on others. Most of the necrotic areas dropped out, but the ring spots, for the most part, either remained unchanged or gradually faded out. Symptoms were prevalent on foliage above the inoculation point, but were few below. The leaves produced from mid-June on were symptomless, so that, in late summer, the trees presented at first glance an almost healthy appearance, though, on closer examination, symptoms could still be seen on the older leaves.

In the second year, a different set of symptoms made their appearance at the beginning of the season, consisting of shiny lines and a trace of mottle on a few leaves, but there was little or no necrotic spotting. Much of the foliage put out up to the end of June was characterized by undulant margins, some rugosity, and more or less twisting of the laminae. As the season progressed, the new leaves developed more normally, and tended to obscure the general symptom picture.

On Myrobalan seedlings.—Although the tissues of buds and stock had united in several instances, so that transmission was possible, no symptoms were observed on Myrobalan seedlings in two years after inoculation.

On Mahaleb seedlings.—The leaves of the two Mahaleb seedlings on which union with the diseased bud had taken place exhibited a very faint mottle early in the first season and a variety of patterns, such as oak-leaf, coarse

lines and rings, and fine confluent rings, later in the summer. The latter symptoms reappeared on only one of the seedlings in the second year. A third Mahaleb seedling, on which the bud died shortly after insertion, remained symptomless both years.

DISCUSSION

Experience in recent years has shown that some plum and sweet cherry varieties and especially the sour cherry variety, Montmorency, can and frequently do carry virus without showing recognizable symptoms. Peaches, on the other hand, have so far been found to be free from masked viroses in nature, though it has been shown experimentally that they can act as carriers of certain viruses after having "recovered" from the initial phase of the disease. This characteristic of certain varieties of carrying a virus symptomless has to be taken into account in all experiments with virus diseases of stone fruits, however, to date, there has been no positive evidence that a masked virosis in a carrier has modified to any great extent the symptoms induced by another virus transmitted to that host. Consequently, symptoms manifested by a variety after, but not before, inoculation can be considered to be the result of that inoculation.

The plum varieties tested so far, as well as the Myrobalan stock, have been little affected by tatter leaf. Any indications of transmission have been so tantalizingly indefinite that transfers from inoculated plum varieties back to cherry and peach will have to be made for confirmation.

There were some apparent discrepancies in the symptom picture on peaches with regard both to the early and to the mid-season symptoms. It should be noted that the rings and chlorotic markings, which, occurring only at the beginning of the first season after inoculation, may be regarded as the acute phase of the disease, *cf.* McKinney and Clayton (8), were found on the nursery stock but not on the double-budded seedlings which were cut back. It has also been observed, in connection with a number of other stone-fruit viruses under investigation, that the acute symptoms which they normally induce on unpruned peach seedlings have been suppressed by cutting the seedling back to the bud. There is no reason to believe that the movement of the tatter-leaf virus in the host differs essentially from that of other viruses which is considered to be in the direction of the flow of the main stream of elaborated foods (1, 2). Accordingly, after inoculation in the fall, the virus would tend to move out into the tissues in the immediate vicinity of the inserted bud and possibly below it, and to remain there until carried to the upper part of the tree when movement of food materials and growth are resumed in the spring. Indeed, the distribution of symptoms in Montmorency suggests that little movement of the virus takes place until spring. McKinney and Clayton (8) consider that the developmental stage at which tissue becomes infected tends to determine the type of disease reaction. If, as suggested by Valleau (10) in connection with ring spot of tobacco, the acute phase is due to the infection of already differentiated tis-

sues and the chronic phase follows invasion of undifferentiated embryonic tissue, it would appear that the tatter-leaf virus did not move out into the upper buds of either seedling or larger tree until after the first leaves were partly formed. In support of this view was the tendency for the acute symptoms to be located in the basal three-quarters of affected leaves rather than in the tips. Cutting back the inoculated seedlings, besides circumventing this development, either forces into growth already infected buds close to the graft or induces the production of adventitious shoots from infected tissue. The early appearance of chronic symptoms in the first season on cut-back seedlings can be accounted for by the development of shoots from infected meristems, supplemented by the comparatively rapid multiplication of the virus during the very vigorous growth induced by the cutting-back operation. On the other hand, in the larger trees where chronic symptoms were delayed until the second season or later, the slower accumulation of the virus to the degree of concentration and distribution necessary for symptom expression, was probably due to the relatively smaller dosage of inoculum, only one or two diseased buds being used in inoculation, and in some measure to the less rapid multiplication of the virus incident to less vigorous growth.

Some differences in the reaction of sweet cherry varieties to tatter leaf were indicated. Napoleon appeared to be the least susceptible, exhibiting chlorotic ring, line, and other patterns, and comparatively little necrosis and tattering. Bing, on the other hand, was more affected by tattering than was Black Tartarian, particularly with respect to the size of the necrotic areas on individual leaves. While Black Tartarian appears to occupy an intermediate position of susceptibility, its behavior after inoculation has been somewhat anomalous, both in the severity and extent of tattering and in the rate at which infection spread through individual trees. It may well be that the differences observed should be attributed to the time of year at which infection took place rather than to the vagaries of the variety itself. The orchard tree, in which violent symptoms of tattering appeared over about a third of the tree in the first season and which was almost completely infected the next year, was inoculated in June when active growth was in progress. All other Black Tartarian trees and those of other cherry varieties were inoculated in late July or early August after growth had slowed down. Symptom expression in these trees was restricted the following spring to shoots near the point of inoculation and at least two more growing seasons were required for the disease to permeate each tree. It is therefore reasonable to suggest that both the movement and the multiplication of the virus in the host were more extensive after the early summer infection than after the later ones.

The rôle of foliar necroses and lacerations in the symptom expression of tatter leaf on sweet cherries is not entirely clear at present. On the grounds that the tree from which the virus was originally obtained had no such symptoms and may have had the disease for a number of years, it may be argued that the tatter-leaf condition is part of the acute phase. A similar conclusion

is suggested by the almost complete absence of tattering both on the sweet cherry seedling inoculated in 1941 and on the Black Tartarian budded thereon, if it be assumed that cutting back suppresses acute symptoms in sweet cherries as it does in peaches. Furthermore, the evidence so far obtainable from the inoculations of nursery stock and of larger trees, while inconclusive, is not incompatible with the theory. On the other hand, recurrence of tattering on the Black Tartarian inoculated in June, 1940, indicates that the so-called acute symptom, though gradually waning, may have almost the persistence of a chronic one. It seems probable that the time of year at which infection occurred and possibly a higher level of virus content may have been factors in prolonging the production of an acute symptom. Whether or not the necroses and lacerations are shown to be the acute symptoms and the non-necrotic patterns, the chronic ones, both types are manifest on sweet cherry only on leaves produced in the early part of the summer. Additional data are necessary to determine how much this seasonal peculiarity is due to external factors, as for example temperatures during leaf development (7), and how much to internal factors, such as the rate of movement and multiplication of virus within the host.

The etched rings and necrotic spotting which constitute the acute symptoms of tatter leaf on Montmorency, bear considerable resemblance not only to the acute symptoms of the more virulent strains of prune dwarf (12), but also to those of necrotic ring spot of sour cherry (3, 6) on the same host. This similarity of symptoms can be explained by assuming either that each of the diseases in question is caused by one of three closely related single viruses, or that one or more is caused by a virus complex, one component of which is common to all three, or that Montmorency can react with much the same response to more than one distinct virus. Though the problem of possible relationships thus posed cannot be fully resolved from the data at present available, some deductions can be made. The distinct differences between the symptoms of tatter leaf and those of prune dwarf on other varieties of the range of differential hosts is an argument against the close relationship of the causal viruses and in favor of the theory of like host response to unlike causes, provided of course that a virus complex is not involved in one or both diseases. Up to the present, differential symptom expression on the range of hosts used in the experiment and particularly on the peach, leads to a similar conclusion, regarding tatter leaf and necrotic ring spot, which is supported by the following piece of circumstantial evidence. Subinoculation to clonal yearling Italian prune trees from the Montmorency, Napoleon, and Black Tartarian nursery stock, to which tatter leaf had been previously transmitted, demonstrated that the Montmorency carried a second virus which was not present in the other two varieties and which in all probability was one of the forms of necrotic ring spot commonly occurring in masked form in Montmorency. Since the inoculation with tatter leaf induced striking symptoms on an already diseased Montmorency, the introduced virus was evidently not the same as the inherent one. On the other hand, one of

two Black Tartarian trees inoculated with necrotic ring spot from sour cherry had, in two seasons, leaves with symptoms resembling those of tatter leaf, though, on the whole, the sweet cherry was much less affected by the necrotic-ring-spot virus than by the tatter-leaf virus. Hildebrand also reported (6, 11) that the sour cherry ring spot induced tatter-leaf symptoms on sweet cherry. It is possible that the necrotic-ring-spot virus he used was more closely related to the tatter-leaf virus than the Canadian strain seems to have been.

In so far as prune dwarf, tatter leaf, and necrotic ring spot of sour cherry can be separated as three distinct viroses, on the basis of their symptom expression on a common range of differential hosts, it is necessary to postulate the existence of at least three distinct viruses or virus strains, whether they act singly or as part of a complex. If they are related and how closely they are related are vexed questions which must await the outcome of further research.

SUMMARY

Tatter leaf of sweet cherry is a virus disease named for its most striking symptom on that host, and has been found in several orchards of the Niagara Peninsula in Ontario. One strain of the causal virus has been transmitted to a range of hosts comprising several varieties of plums, peaches, and sweet and sour cherries.

The disease has little apparent effect on plum varieties, some of which react with an indistinct mild mottle on early leaves.

On peach, the acute symptoms (slight superficial bark necrosis, ring patterns, and chlorotic markings) appear at the beginning of the first growing season after inoculation. Subsequently, usually in the second year, chronic symptoms including faint mosaic and oak-leaf patterns, dullness and premature aging of the upper leaf surface, fine red pin-spotting, and occasionally red rim, fawn necroses, are to be found on leaves scattered over the tree. Acute symptoms are suppressed in favor of the chronic symptoms when seedlings inoculated in late summer are cut back to the bud the following spring.

The first symptoms to appear on Black Tartarian cherry trees the spring after inoculation are fine brown lines circumscribing interveinal areas which soon become necrotic and drop out. Faint yellowish mottling and oak-leaf patterns also occur. Symptom expression appears to be confined to leaves emerging early in the growing season and to vary in intensity and extent with the time of year at which infection takes place, becoming less intense and more restricted in distribution the later the infection. Necrosis and laceration affect Napoleon to a lesser and Bing to a greater degree than Black Tartarian.

Fine etched rings and necrotic spotting form the acute phase, and undulations, rugosity, and twisting of the leaf laminae the chronic phase on the sour cherry variety, Montmorency. The acute symptoms resemble those of ne-

crotic ring spot and some strains of prune dwarf on that variety, but the relationships, if any, of the causal viruses are as yet a matter of conjecture.

DOMINION LABORATORY OF PLANT PATHOLOGY,
ST. CATHARINES, ONTARIO, CANADA.

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